Crosstalk Between the Gut and Brain: Importance of the Fecal Microbiota in Patient With Brain Tumors

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Background: Variations in the gut microbiota may affect the metabolism, inflammation and immune response of the host. Microbiota dysbiosis has been extensively investigated in neurological disorders and diseases of the central nervous system (CNS). However, the alterations of the gut microbiota in patients suffering from brain tumors and the associations of the gut microbiota with these diseases remain unknown. Herein, we investigate the alterations of the gut microbiota community in patients with brain tumors and the associations between the two and further explore microbial markers used for the diagnosis of brain tumors.

Methods: In our study, we recruited 158 participants, consisting of 101 brain tumor patients (65 benign and 36 malignant cases) and 57 age- and sex-matched healthy controls (HCs). We characterized the gut microbial community by using 16S rRNA gene amplicon sequencing and investigated its correlations with clinical features.

Results: The results showed remarkably less microbial ecosystem richness and evenness in patients with brain tumors than in HCs. The gut microbiota community structure underwent profound changes in the brain tumor group, including an increase in the abundances of pathogenic bacteria, such as *Fusobacteriota* and *Proteobacteria* and a reduction in the abundances of probiotic bacteria, such as *Bifidobacterium* or *Lachnospira*. Moreover, our study indicated more significant correlations and clustering of pathogens in the malignant brain tumor group. Furthermore, a biomarker panel was used to discriminate the brain tumor patients from the healthy controls (AUC: 0.77). Kyoto Encyclopedia of Genes and Genomes (KEGG) annotation revealed an accumulation of harmful metabolites and disorders of the basic physiological pathways in the brain tumor group.

Conclusions: Our study revealed that brain tumor patients may possess divergent host-microbe interactions from those of healthy controls, especially in malignant brain tumor
The human gut, one of the most dynamic niches, harbors trillions of microbiome constituents, including bacteria, fungi and other species (Milani et al., 2017). The microbiome plays an essential role in the maintenance of host homeostasis by regulating several important physiological processes, including nutrient metabolism, maturation, and stimulation of angiogenesis (Zmora et al., 2019). Moreover, accumulating evidence suggests that nutrient metabolism, maturation, and stimulation of angiogenesis regulating several important physiological processes, including essential role in the maintenance of host homeostasis by the neuromethylation patterns (Gusyatiner and Hegi, 2018). The loss of Mutations targeting isocitrate dehydrogenase 1/2 (IDH 1/2) have formation of meningiomas and cranial nerve schwannomas other species (Milani et al., 2017). The microbiome plays an environmental and nongenetic endogenous factors are closely related to several diseases and neurological disorders in the CNS.

The gut microbiome and its metabolites, such as short-chain fatty acids (SCFAs) and neurotransmitters, can change the CNS microenvironment, adjust immune responses, and disturb the endocrine system (Dalile et al., 2019). Epidemiological studies have further demonstrated that dysbiosis in microglial activation and amino acid metabolism pathways could be triggered by an unbalanced intestinal flora and result in extensive tissue remodeling and disruption of the antitumoural immune response in the brain tumor microenvironment. In addition, one animal study further revealed that alterations in microbial composition could promote glioma pathophysiology in rats (Patrizz et al., 2020). Although increasing evidence suggests a close connection between brain tumors and abnormalities in gut microbial function, evidence from only animal or epidemiological studies is insufficient to identify the exact correlations and mechanisms in humans. Hence, understanding of the microbial composition and the composition of metabolites in the gut microbiota in brain tumor patients is still unclear. Therefore, an intense interest in characterizing the functional intestinal flora in brain tumor patients has arisen.

In this study, we applied 16S rRNA gene sequencing to fecal samples from healthy controls and brain tumor patients to characterize the intestinal microbial community and explore potential microbial biomarkers for early diagnosis and potential targeted therapy.

**METHODS**

**Research Design and Participants**

In this research, a total of 158 participants were recruited by the Department of Neurosurgery, Clinical Medical College of Yangzhou University (Jiangsu, China) from June 2020, to August 2021, including 101 patients with brain tumors and 57 healthy controls. All participants received conventional medical and stool examinations. Their clinical data were recorded, including age, sex, body mass index (BMI), etc. All the patients and healthy controls were Jiangsu Han Chinese, with similar compositions of metabolites in the gut microbiota in brain tumor patients has arisen.

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or prebiotics within two months before sampling. Our research was approved by the Ethics Committee of the Clinical Medical College of Yangzhou University (2021ly-007-1), and the present study was registered at the Chinese Clinical Trial Register (ChiCTR2100044256). Written informed consent was obtained from the brain tumor patients and the healthy controls.

Fecal Sample Collection and DNA Extraction For Microbiome Analysis

All fecal specimens were collected by sterile cotton swabs within 2 hours of hospital admission, immediately placed in a proprietary preservation solution and then immediately stored at -80°C until further testing. Genomic DNA was extracted with a PowerMax extraction kit and stored at -80°C. The quantity and quality of DNA were determined by a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

16S rRNA Amplicon Pyrosequencing

PCR amplification of the bacterial 16S rRNA gene V4 region was performed using the forward primer 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and the reverse primer 806R (5'-GGACTACHVGGGTWTCTAAT-3'). Sample-specific paired-end 6-bp barcodes were incorporated into the TruSeq adaptors for multiplex sequencing. Thermal cycling consisted of initial denaturation at 98°C for 30 s, followed by 25 cycles of denaturation at 98°C for 15 s, annealing at 58°C for 15 s, and extension at 72°C for 15 s, with a final extension at 72°C for 1 min. PCR amplicons were purified by AMPure XP Beads (Beckman Coulter, Indianapolis, IN) and quantified using a PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA). After the individual quantification step, amplicons were pooled in equal amounts, and paired-end 2×150 bp sequencing was performed using the Illumina NovaSeq6000 platform.

Sequencing Data Analysis

The paired-end reads were assigned to respective samples using specific barcodes, which were cut off with primer sequences together. Then, the truncated reads were merged using the FLASH (Fast Length Adjustment of SHort reads) tool. The Quantitative Insights Into Microbial Ecology (QIIME, v1.9.0) pipeline was employed to filter the raw tags, and then high-quality clean tags were obtained. Chimeric sequences were filtered using Vsearch (Version 2.4.4) software. Sequences with similarity thresholds greater than 97% were allocated to one operational taxonomic unit (OTU) by using CD-HIT software online (v4.6.1). Classification of representative sequences for each OTU was performed, and the taxonomic data were assigned to each sequence by the Ribosomal Database Project (RDP) classifier 2.10.1. To determine the phylogenetic differences in dominant OTUs, multiple sequence alignments were carried out using Python Nearest Alignment Space Termination (PyNAST) software. Sequences were aligned for phylogenetic analysis after the taxonomic assignment of OTUs. OTU-level alpha diversity indices (Shannon, Simpson, Chao1) were calculated to evaluate the complexity of microbial diversity in each sample using the OTU table in QIIME. Beta diversity analysis was performed to investigate the structural variation in microbial communities across samples using UniFrac distance metrics, and the data were visualized via principal coordinate analysis (PCoA) and nonmetric multidimensional scaling (NMDS). The analysis of similarities (ANOSIM) was calculated to evaluate the statistical significance. The influence of differentially abundant taxa was evaluated using linear discriminant analysis (LDA) coupled with the effect size (LEfSe) method. KEGG (Kyoto Encyclopedia of Genes and Genomes) and the Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) database were used to conduct pathway enrichment analysis. The output file was further analyzed using Statistical Analysis of Metagenomic Profiles (STAMP, v2.1.3) software. BugBase is a tool for measuring high-level phenotypes in the microbiome (https://bugbase.in/).

Statistical Analyses

Clinical and sequencing data analyses were mainly performed using SPSS (v19.0) and R packages (v3.2.0). The continuous variable data are displayed as the mean ± standard deviation, and an independent t test was conducted to calculate the differences between groups. Moreover, we further compared microbial abundance and diversity in fecal samples between brain tumor patients and healthy controls using the Wilcoxon rank-sum test. The differences in the UniFrac distances for pairwise comparisons between groups were determined using Student’s t test or the Monte Carlo permutation test. The genus-level heatmaps were also drawn on the basis of the nonparametric Wilcoxon test (q < 0.1). In addition, the chi-square test or Fisher’s exact test was also used for categorical variables. Finally, p values adjusted using an FDR (false discovery rate) of less than 0.05 were considered statistically significant in the comparisons of groups.

RESULTS

Summary of Clinical Parameters

In the current study, we enrolled 101 patients with brain tumors (T group), as well as 57 matched healthy controls (C group). The clinical variables of the two groups were completely matched (Table 1), indicating that no established confounding factors affected group discrimination prior to the design of this experiment. Moreover, to determine whether there were differences in the gut microbiota associated with different brain tumors, we divided the brain tumors into benign brain tumors (BBTs, mainly meningiomas and pituitary tumors, 65 cases) and malignant brain tumors (MBTs, gliomas and metastatic brain tumors, 36 cases) and included the 57 matched healthy controls (HCs) to conduct the subgroup analysis.

Diversity of the Gut Microbiota of Brain Tumor Patients

The gut microbiota was evaluated using 16S rRNA sequencing. A total of 19,948,889 high-quality 16S rRNA reads were identified,
TABLE 1 | Descriptive data of included subjects in the study.

| Characteristics | Brain tumours (T) (n = 101) | Benign brain tumours (BBT) (n = 65) | Malignant brain tumours (MBT) (n = 36) | Healthy Controls (C) (n = 57) | BBT vs. MBT T vs. C p | p |
|-----------------|-------------------------------|-------------------------------------|--------------------------------------|-------------------------------|-----------------------|---|
| Age (Mean ± SD) | 57.34±10.68                  | 55.92±10.50                         | 59.89±9.80                          | 57.05±11.46                  | 0.462                 | 0.765 |
| Gender          |                               |                                     |                                      |                               | 0.322                 | 0.925 |
| Female          | 57                            | 34                                  | 23                                   | 33                            |                       |    |
| Male            | 44                            | 31                                  | 13                                   | 24                            |                       |    |
| BMI             | 23.91±3.24                   | 24.12±3.17                          | 23.53±3.38                          | 23.71±3.47                   | 0.462                 | 0.628 |
| Smoking         |                               |                                     |                                      |                               | 0.145                 | 0.417 |
| Absence         | 72                            | 46                                  | 26                                   | 44                            |                       |    |
| Presence        | 29                            | 19                                  | 10                                   | 13                            |                       |    |
| Drinking        |                               |                                     |                                      |                               | 0.786                 | 0.505 |
| Never           | 59                            | 40                                  | 19                                   | 36                            |                       |    |
| <1 standard drink per day | 29 | 19 | 10 | 17 |                       |    |
| ≥1 standard drink per day | 13 | 6 | 7 | 4 |                       |    |
| Hypertension    |                               |                                     |                                      |                               | 0.766                 | 0.485 |
| Negative        | 78                            | 55                                  | 23                                   | 47                            |                       |    |
| Positive        | 23                            | 10                                  | 13                                   | 10                            |                       |    |
| Diabetes        |                               |                                     |                                      |                               | 0.421                 | 0.254 |
| Presence        | 69                            | 45                                  | 24                                   | 46                            |                       |    |
| Excrement regularity | 21 | 14 | 7 | 5 |                       |    |
| Yes             | 82                            | 58                                  | 24                                   | 47                            |                       |    |
| No              | 19                            | 7                                   | 12                                   | 10                            |                       |    |
| Tumor location  |                               |                                     |                                      |                               | 0.024                 |        |
| Left            | 48                            | 38                                  | 10                                   | –                             |                       |    |
| Right           | 45                            | 27                                  | 18                                   | –                             |                       |    |
| Diffuse         | 8                             | 8                                   | 8                                    | –                             |                       |    |
| Tumor maximum diameter (Mean ± SD) | 55.3±15.2 | 50.5±14.3 | 61.2±16.4 | – | <0.01 |

with a median read count of 130,521 (ranging from 95356 to 138015) per sample. After taxonomic assignment, 5469 operational taxonomic units (OTUs) were obtained, including 12 phyla, 59 families and 147 genera of gut microbes (Figure 1A). Alpha diversity indices, which were analyzed using sampling-based OTUs, were further calculated to evaluate the differences in the microbial diversity between each group. The results indicated that the gut microbial alpha diversity of brain tumor patients was significantly lower than that in healthy subjects in terms of the Shannon, Simpson and Chao1 indices (Figures 1B–D; p=0.0001, 0). The subgroup analysis showed no difference in alpha diversity between the BBT and MBT groups (Figures 1E–G; p=0.32, 0.41, 0.64). The beta diversity was evaluated to illustrate the differences in the structural variation in microbial communities between groups. Principal coordinate analysis (PCoA) based on unweighted UniFrac distance showed that the gut microbiota of brain tumor patients clustered somewhat separately from that of healthy subjects (Figure 1H; PC1 = 0.0049, PC2 = 0.0066). Nonmetric multidimensional scaling (NMDS) analysis based on Bray–Curtis distances between groups revealed a significant deviation in microbial abundances (STRESS=0.1103<0.2, Figure 1I). The subgroup analysis showed no obvious difference in beta diversity between the MBT group and the BBT group. (PC1 = 0.064, PC2 = 0.065, Figure 1K; STRESS=0.3067>0.2, Figure 1L). The results of similarities analysis (ANOSIM) revealed that each group has good representativeness with small intragroup differences, so the grouping is meaningful between the brain tumor group and the healthy group (ANOSIM, r=0.166>0, p=0.001, unweighted UniFrac, Figure 1J), and the same results were obtained between the MBT and BBT groups (ANOSIM, r=0.123>0, p=0.001, unweighted UniFrac, Figure 1M).

Alterations in the Composition of the Fecal Microbiota in Brain Tumor Patients

To investigate the intestinal microbial community features of brain tumor patients, the relative taxon abundance of the microbiota was compared among different groups, and considerable variability was observed.

At the phylum level, the brain tumor group had a higher abundances of Bacteroidetes (45.6% vs. 36.9%, p=0.004), Fusobacteria (2.13% vs. 0.37%, p<0.001) and Proteobacteria (8.23% vs. 4.77%, p=0.005) and lower relative abundances of Firmicutes (41.58% vs. 54.31%, p<0.0001), Actinobacteria (1.22% vs. 1.96%, p=0.0005) than those of the healthy controls. Hence, the Firmicutes/Bacteroidetes (F/B) ratio was decreased significantly in the tumor group (p<0.001, Figures 2A–C). Moreover, Fusobacteria and Proteobacteria showed a higher abundance in the MBT group than in the BBT group (Figure 2F).

At the family level, the brain tumor-enriched species included Bacteroidaceae (p=0.027), Fusobacteriaceae (p<0.001) and Enterobacteriaceae (p<0.001), whereas Bifidobacteriaceae (p<0.001), Lachnospiraceae (p=0.002), and Akkermansiaaceae (p=0.042) were more prevalent in the healthy control group (Figure 2D and Supplementary Table 1). Moreover, there was an obviously higher abundance of Enterobacteriaceae and a lower abundance of Lachnospiraceae in the MBT group than in the BBT group (Figure 2G).
The genus-level characterization results were more complicated, and the relative abundances of the top 60 dominant taxa in all samples were further calculated by clustering analysis and displayed in the heatmap shown in Supplementary Figure 1. After independent t test and Wilcoxon rank-sum test, a total of 31 genera were identified as significantly differentially abundant between each group. Of these discriminatory taxa, *Bacteroides* (*p*=0.027), *Escherichia/Shigella* (*p*<0.001), *Fusobacterium* (*p*<0.001), *Sutterella* (*p*=0.047), and *Ruminococcus gnavus* group (*p*<0.001) showed...
visibly higher abundances in the tumor patient group than in the healthy control group (Figure 2E and Supplementary Table 2). Moreover, the abundances of the genera *Roseburia* and *Megamonas* were lower, and the abundance of *Escherichia*/*Shigella* was higher in the MBT group than in the BBT group (Figure 2H).

**Potential Microbial Biomarkers for Patients With Brain Tumors and Healthy Controls**

Linear discriminant analysis (LDA) effect size (LEfSe) analysis was performed to generate a cladogram to identify the specific microbiota of the brain tumor patient group and the healthy controls.
control group (Figure 3A). A variety of opportunistic pathogens, including *Fusobacterium* (*Fusobacteriaceae, Fusobacteria*), *Enterobacteriaceae* (*Enterobacterales*) and *Escherichia/Shigella*, were all significantly overrepresented in brain tumor patients ($p < 0.01$, Wilcoxon rank-sum test; LDA, log 10 $> 5$), whereas *Parasutterella, Bifidobacterium* (*Bifidobacterales, Actinobacteria*) and *Lachnospira* were significantly enriched (LDA, log 10 $> 7$) in the healthy controls (Figure 3B). In addition, the unique bacterial biomarkers for different groups (MBT, BBT, HC) are illustrated in Figures 3C, D. We found abundant common biomarkers for

**Figure 3** | Differential enriched bacterial taxa between different groups. Linear discriminant analysis (LDA) integrated with effect size (LEfSe) was used to identify the taxa with the greatest differences in abundance between different groups. Cladogram indicating the phylogenetic distribution of microbiota correlated with the T or C groups (A). Histogram of the LDA scores computed for differentially abundant taxa between T and C groups (B). Meanwhile, the enriched taxa in the malignant brain tumours, benign brain tumours and the healthy were also displayed in the cladogram (C), and the differences in abundance between the malignant and benign brain tumours (D). The central point represents the root of the tree (microorganism), and each ring represents the next lower taxonomic level. The diameter of each circle represents the relative abundance of the taxon, only the taxa satisfied an obvious LDA threshold value of $>2$ are presented.
healthy subjects, while the genera *Roseburia* and *Hungatella* were enriched in the BBT patients, and *Escherichia/Shigella* and *Fusobacterium* were the most abundant in the MBT patients.

**The Gut Flora-Based Signature Discriminates Tumor Patients From Healthy Subjects**

Several differentially abundant taxa of the gut flora were revealed in the brain tumor group, and then we assessed the possible value of using six abundant genera, including *Bifidobacterium*, *Bacteroides*, *Lachnospira*, *Fusobacterium*, *Parasutterella*, and *Escherichia/Shigella*, as biomarkers. The differential features of these genera showed obvious intergroup changes (Figures 4A–F). Then, each of six differentially abundant bacteria was used to generate receiver operating characteristic (ROC) curves to predict classification of the groups, and an area under the curve (AUC) ranging from 0.55 to 0.65 was obtained (Figure 4G). In addition, we discovered that combining all six genera significantly improved the predictive performance (AUC: 0.77). Hence, this microbial biomarker panel could be used to discriminate brain tumor patients from healthy controls.

**The Functional Profile of the Intestinal Flora in Patients With Brain Tumors Was Changed**

An imbalanced microbiota could induce systematic metabolic dysfunction, whereas metabolic dysregulation could in turn alter intestinal flora composition (Song et al., 2020; Fan and Pedersen, 2021). To investigate the metabolic and functional alterations in gut microbial communities, each OTU was aligned into the PICRUSt built-in reference database and the Carbohydrate-Active enZymes (CAZy) database. The analysis based on the PICRUSt and CAZy databases identified the top 20 KEGG pathways and top 7 CAZys with significantly differential abundance between groups. As
presented in Figures 5A, B, the pathways of bacterial motility, membrane transport, biosynthesis of essential substances and energy metabolism were less abundant, while the pathways involved in the accumulation of toxic substances were more abundant in the tumor group than in the control group. According to the results of the gene annotation performed based on the KEGG Orthology database, the clustering of gut metabolic modules mainly involved the degradation of neurotransmitters, such as amino acids or their precursors, and the brain tumor group showed a significantly higher abundance than that of the healthy control group (Figure 5C). Subgroup analysis showed that the MBT group had a higher degree of change than that in the BBT group in many basic KEGG pathways (Figure 5D).

The Associations Between the Various Bacterial Communities Were Complicated

In the tumor group, there were conspicuous dynamic relationships among the 10 phyla, including 11 positive relationships and 18 negative relationships. The abundances of *Firmicutes* were all strongly negatively correlated with the abundances of *Bacteroidetes*, *Proteobacteria* and *Fusobacteria*. The abundance of *Fusobacteria* was positively correlated with...
the abundances of Bacteroidetes and Proteobacteria but negatively correlated with the abundances of Verrucomicrobia and Actinobacteria (Figure 6A). To explore the underlying reasons for these relationships, Bugbase software was used to identify the phenotypic characteristics of the microbiota, and the results were as follows in the comparison of the brain tumor group and the healthy group: the number of gram-positive bacteria was evidently lower \( (p=0.00025) \), and the number of gram-negative bacteria \( (p=0.0011) \) and opportunistic pathogens \( (p=0.00012) \) showed the opposite trend, while there was no significant difference in biofilm formation \( (p=0.19) \). (Figures 6B–F);

**DISCUSSION**

The gut harbors a broadly diverse microbiota that can produce an extremely wide range of small molecules that affect several vital pathways associated with immune balance and the homeostasis of neurological function (Cryan et al., 2019; Song et al., 2020). In recent years, accumulating evidence has revealed that the gut microbiota and its metabolites contribute to neurological disorders and tumorigenesis in various systems (Wong and Yu, 2019; Di Modica et al., 2021). The characteristics and regulation of the gut microbiota have gradually become emerging tools to diagnose and treat tumor patients.
Recent studies have suggested that a strong relationship between the microbiota and brain tumors may exist. Yuqi Wen et al. proved that the oral microbiota could be a useful biomarker to distinguish glioma patients from healthy individuals (Cryan et al., 2020). A study from Yingying Lyu et al. (Patrizz et al., 2020) also revealed that the gut microbiota could regulate the immune environment of gliomas by the gut-brain axis. However, most related studies have a small sample size. In our present study, we recruited 101 patients with brain tumors (which could be divided into BBT and MBT groups) and 57 matched normal controls to explore the underlying relationships between the gut microbiome and brain tumors. The results of our comparison of the brain tumor and healthy groups suggested that the brain tumor group presented a microbial ecosystem with low density and loss of microbial diversity. The alpha and beta diversity analyses showed that the microbial structure of brain tumor patients was also obviously different from that in the healthy group. Moreover, we investigated the differences between the BBT and MBT groups. However, the results indicated similar alpha and beta diversity between the BBT and MBT groups. In addition to microbial diversity, we focused on microbial gene functions and on the development of a potential biomarker panel that could be used to distinguish brain tumor patients from healthy controls and aimed to identify significant differences in the gut microbiota of BBT and MBT patients. Hence, this study may provide evidence of substantial changes in the gut flora of benign and malignant brain tumor patients.

Significant changes in brain tumor-associated gut microbiota compositions were observed in our research. The gut microbial composition may be obviously relevant to brain tumor malignancy. For example, at the phylum level, the intestinal microbiota showed the lowest abundance of Firmicutes in the MBT group and the lowest abundance of Actinobacteria in the BBT group, unlike those in the healthy group. Several studies have shown that Firmicutes can regulate anti-inflammatory activities and apoptosis by producing butyrate (Dalile et al., 2019; Tran and Mohajeri, 2021; Qu et al., 2021). Actinobacteria are valeric acid-associated microbes and have also been demonstrated to be depleted in colorectal tumors (Yang et al., 2019). In the MBT group, we revealed increased abundances of Fusobacteria and Proteobacteria. Fusobacteria, which are opportunistic pathogens, have been reported to promote tumor cell proliferation by regulating T regulatory cells and the activation of autophagy (Chen et al., 2020). In addition, most genera of Proteobacteria have been classified as gram-negative pathogens (Vaz-Moreira et al., 2017; Guan et al., 2018). The abnormal increase in Proteobacteria abundance indicates that the intestinal epithelial barrier is broken and more susceptible to infection (Yoo et al., 2020). In the BBT group, we found that Bacteroidetes was especially enriched. Bacteroidetes has been repeatedly reported to be essential for the host by performing metabolic conversion. The imbalanced Bacteroidetes abundance could induce neuroinflammation by the production of excessive propionate (Li et al., 2019). This chemical substance has a dose-regulated influence on brain cell neurotoxicity and microglial activation (El-Ansary et al., 2012). Moreover, the excessive abundance of Bacteroidetes bacteria in brain tumors reduced the ratio of Firmicutes to Bacteroidetes. A lower F/B ratio is associated with the dysbiosis of host systemic inflammation and immunity by decreasing the concentration of circulating SCFAs (Dalile et al., 2019; Liu et al., 2019; Liu et al., 2019). Hence, a generally imbalanced gut microbial ecosystem associated with the degree of malignancy was revealed in brain tumor patients.

Various additional pathogenic bacteria were demonstrated to be enriched at the family and genus levels, indicating that the characteristics are more complicated and significantly varied in patients with brain tumors. For example, the abundance of the genus Sutterella was increased in the brain tumor group. A study suggested that Sutterella could drastically reduce the diversity of the microbial ecosystem by degrading IgA and promoting tumor progression (Kaakoush, 2020). Except for that of Sutterella, the abundances of almost every genus revealed a great increase in brain tumor patients, and this microbial signature was then proven to be a tumor biomarker with different tumor-promoting effects. Contrary to the results of previous studies that identified Bacteroides as a probiotic for enhancing anti-CTLA4 immune checkpoint efficacy (Wexler, 2007; Liu et al., 2019), excessive Bacteroides abundance could be neurotoxic (Wexler, 2007; Cattaneo et al., 2017). In addition, it has been reported that Bacteroides and Clostridia could possess complicated pathways to metabolize tryptophan and modulate this metabolite by regulating TPH1 (a key rate-limiting enzyme) (Yano et al., 2015; Chen et al., 2021; Taverniti et al., 2021). The abundance of serotonin could have a bidirectional effect on brain tumor growth (Dehghahi et al., 2020). The genera Escherichia/Shigella and Fusobacterium were also revealed as biomarkers for malignant brain tumors. Both of these genera have been proven to be associated with cancers. They may promote MBT proliferation by increasing the levels of neurological inflammation and regulating the expression and activity of DNA methyltransferase, respectively (Xia et al., 2020). In the BBT group, the genus Roseburia, which has potential for use as a probiotic for the treatment of diseases, was significantly enriched. Its ability to modulate the brain functional connectivity pattern by tumor necrosis factor-alpha (TNF-α) may be the reason for this observation. Moreover, many probiotic bacteria were also revealed as biomarkers for the healthy group. The genera Bifidobacterium and Parasutterella are the core components of the gut microbiota, and the loss of these genera could contribute to the imbalance of the immunity, neurohormone and metabolic systems (Kang et al., 2017; Jena et al., 2018). Moreover, both the Clostridia and Lachnospira genera could inhibit tumor proliferation by producing butyric acid (Calvo-Barreiro et al., 2021). All the evidence suggests that MBT patients possess more abundant pathogens and that specific constituents of the gut microbiome may be applied to diagnosis or used as therapeutic targets and even for fecal microbiota transplantation. Furthermore, the alteration of the gut bacterial population associated with brain pathology, which can be assessed using metrics such as alpha and beta diversity, may provide potential predictive information for tumor progression and recurrence.
In addition, we should pay attention not only to the specific constituents of the gut microbiome in brain tumor patients but also to the interactions among them. We speculate that the underlying dynamic connections among the microbiome may play an essential role in tumor growth. For example, the lipopolysaccharide of gram-negative bacteria (mostly from Proteobacteria) could promote the proliferation of facultative anaerobic pathogens by providing them with energy (Maldonado et al., 2016; Yoo et al., 2020). This relationship suggests a disruption in the oxygen levels in the intestinal environment, which may explain the increased abundance of Enterobacteriaceae and the reduction in the abundance of gut strict obligate anaerobes, including Firmicutes and Akkermansiaceae (Yoo et al., 2020). Then, the increased Enterobacteriaceae abundance could induce neutrophil transepithelial migration and the depletion of SCFA-producing bacteria (e.g., Bifidobacteriaceae and Clostridiaceae genera). Moreover, the depletion of SCFA-producing bacteria may inhibit their ability to limit the colonization of Enterobacteriaceae via intestinal pH reduction (Behnsen, 2017; Yoo et al., 2020), which is shown in Figure 6.

It is known that an imbalanced microbial structure can induce dramatic metabolic disorders. For example, the levels of SCFAs generated by various bacteria, such as Bifidobacterium and Clostridium, may be decreased in MBT and BBT patients. By upregulating the levels of tight junction proteins, SCFAs can regulate the permeability of the gut tract and promote the release of many cytokines and molecules in the blood circulation and CNS (Feng et al., 2018). SCFAs participate in the process of epigenetic modulation by inhibiting histone deacetylase, which promotes the development of MBTs (Tran and Mohajeri, 2021). For immune profiling, SCFAs can activate G-protein-coupled receptors (GPCRs), such as GPR43 and GPR109A, which have a close relationship with microbial cell morphology and growth hormone secretion in pituitary cells (Fernandes et al., 2020; Silva et al., 2020). In addition, SCFAs modify the metabolism and activity of brain tumor-associated immune cells (Bachem et al., 2019). Therefore, dynamic dysbiosis of all the constituents of the gut microbiome, rather than of specific pathogens, could promote tumor development in both MBT and BBT patients.

The analysis of KEGG pathways suggested that the disturbance in the intestinal microflora in brain tumor patients is closely linked with the dysbiosis of several basic physiological processes, mainly including metabolism, cellular processes, and environmental information processing. These data also demonstrated that the synthesis and decomposition of carbohydrates have less abundant roles in the gut microbiota of brain tumor patients. The dysregulation of energy metabolism has been reported to be essential for the pathogenesis of tumors. In summary, these abnormal changes further suggest potential metabolic and immune alterations in brain tumor patients. For example, carotenoids with the ability to activate intracellular signaling cascades could promote inflammation or oxidative stress responses by influencing gene expression and protein translation, and the levels of these compounds were found to be increased in tumor patients (Reboul, 2019). In addition, the inhibition of the bacterial chemotaxis and endocytosis pathways suggests the weakening of bacterial motility and membrane transport, which is consistent with the feature of low gut microbial diversity in brain tumor patients. Moreover, the excessive degradation of amino acids such as arginine and glutamate in the tumor group could result in an increase in neurotransmitter levels (Tran and Mohajeri, 2021) and then activate the uncontrolled proliferation and diffusion of tumor cells (Jiang et al., 2020). Glutamate is an important source of α-KG, which participates in metabolic processes that occurs after IDH1/2 mutation and is associated with the methylation of DNA (Lyu et al., 2021). A study indicated that glutamine could be derived from glutamate and promote tumor autophagy, resulting in increased tumor energy metabolism and eventually contributing to the proliferation of brain tumors (Márquez et al., 2017). In addition, neuroactive metabolites, such as nitric oxide and polyamines, can be derived from arginine, and they can be translocated into the brain and lead to the metastasis and proliferation of brain tumors by modulating spermidine/spermine acetyltransferase or ornithine decarboxylase (Dehghani et al., 2020). Moreover, in the subgroup analysis, we found that the MBT group showed a higher degree of change than that in the BBT group, especially in genetic information processing pathways, such as aminoacyl-tRNA biosynthesis, which suggested that the epigenetic environment is most damaged in the MBT patients.

In summary, the interactions between the gut microbiota and brain tumors are complicated. Unfortunately, current understanding of these interactions is still insufficient. However, all the evidence suggests that brain tumors are fundamentally metabolic diseases and often present with coexisting disorders of the gut microbiota (Liu et al., 2019). Therefore, the perturbations in the microbiome–metabolome interface can be further understood, and several underlying diagnostic or therapeutic targets may be identified in brain tumor patients. Furthermore, it is not definite that the gut microbiota performs its function only by any one of these interactions, and the brain tumor environment and the formation of subtypes may be influenced by alterations in these relationships. We speculate that the process is caused by the tumor-promoting metabolic products and toxins regulating immune and inflammatory responses in the host.

Finally, it has been reported that medicines can affect the progression of tumors by regulating the microbial community (D’Alessandro et al., 2020); moreover, changes in the bacterial community can also improve the efficacy of antitumour drugs and reduce their side effects (Vivarelli et al., 2019), which may provide potential strategies for the treatment of brain tumors. Probiotics or specific synthetic metabolites could be prescribed to combat dysbiosis in tumor patients, especially MBT patients receiving traditional treatments. The relationships between the gut microbiome and brain tumors in this study could provide new inspiration for the investigation of the potential function of the microbiota in the diagnosis and treatment of brain tumors. Hence, larger and more rigorous trials should be designed to explore this essential point.
There were some limitations in our study. First, the nature of the case–control study makes it difficult to dissect the mechanisms and establish a longitudinal view of the relevance of the findings, which is essential to confirm the dynamics of the microbiota and to assess the changes in the intestinal flora of brain tumor patients. Second, no accurate metabolic data were obtained to provide a complete picture of how gut microbiota and corresponding metabolic processes might be impacted during the disease state. Moreover, the subgroup analysis and the associations of brain tumor patients with disease staging were not performed in detail. In addition, to determine the discriminatory power of gut microbial biomarkers, separate test and validation cohorts are required in subsequent studies. The sequencing method has inevitable inherent limitations, such as amplification bias. Finally, it is known that the dysregulation of intestinal bacteria is associated with brain tumors, but we cannot exclude the possibility that microbiome changes may be a passive byproduct of tumor progression. Future research could include corresponding experiments to investigate immune profiling and microbiota-based biomarkers during different tumor stages, establishing a more accurate prediction model for diagnosis and monitoring the stage of diseases.

CONCLUSIONS

Gut microbial dysfunction was demonstrated to yield a group of microbial markers for brain tumor patients. The dysbiosis of several pathogens, including Bifidobacterium, Bacteroides, Lachnospira, Fusobacterium, Parasutterella, and Escherichia/ Shigella, may be an underlying risk for brain tumor development. The use of gut microbiota-based biomarkers could be regarded as a promising noninvasive means to detect brain tumors. However, a characteristic xenograft based on the target microbiome should be regarded as a promising noninvasive means to detect brain tumors. The use of gut microbiota-based biomarkers could be regarded as a promising noninvasive means to detect brain tumors. Future research could include corresponding experiments to investigate immune profiling and microbiota-based biomarkers during different tumor stages, establishing a more accurate prediction model for diagnosis and monitoring the stage of diseases.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: NCBI BioProject - PRJNA807001.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of the Clinical Medical College of Yangzhou University. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

GL, YL and HZ conceptualized, designed, and supervised the project. YL, HJ, and XW conducted the data analysis and wrote the manuscript. HJ, ZW, and QM conducted the experiments. XW, LD, XL, YQ and YH collected the samples. All authors read and approved the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fcimb.2022.881071/full#supplementary-material

REFERENCES

Bachem, A., Makhlouf, C., Binger, K. J., de Souza, D. P., Tull, D., Hochheiser, K., et al. (2019). Microbiota-Derived Short-Chain Fatty Acids Promote the Memory Potential of Antigen-Activated CD8(+) T Cells. Immunity 51, 285–297.e5. doi: 10.1016/j.immuni.2019.06.002

Barnholtz-Sloan, J. S., Ostrom, Q. T., and Cote, D. (2018). Epidemiology of Brain Tumors. Neurol. Clin. 36, 395–419. doi: 10.1016/j.ncl.2018.04.001

Behnse, J. (2017). Protectors of the Neonatal Gut: Clostridia Send Pathogens Packing. Cell Host Microbe 21, 651–652. doi: 10.1016/j.chom.2017.06.002

Calvo-Barreiro, L., Eixarch, H., Cornejo, T., Costa, C., Castillo, M., Mestre, L., et al. (2021). Selected Clostridia Strains From The Human Microbiota and Their Metabolite, Butyrate, Improve Experimental Autoimmune Encephalomyelitis. Neurotherapeutics. 18, 920–937. doi: 10.1007/s13311-021-01016-7

Cattaneo, A., Cattane, N., Galluzzi, S., Provasi, S., Lopizzo, N., Festari, C., et al. (2017). Association of Brain Amyloidosis With Pro-Inflammatory Gut Bacterial Taxa and Peripheral Inflammation Markers in Cognitively Impaired Elderly. Neurobiol. Aging 49, 60–68. doi: 10.1016/j.neurobiolaging.2016.08.019

Chen, Y., Chen, Y., Zhang, J., Cao, P., Su, W., Deng, Y., et al. (2020). Fusobacterium Nucleatum Promotes Metastasis in Colorectal Cancer by Activating Autophagy Signaling via the Upregulation of CARD3 Expression. Theranostics 10, 323–339. doi: 10.7150/thno.38870

Chen, Y., Xu, J., and Chen, Y. (2021). Regulation of Neurotransmitters by the Gut Microbiota and Effects on Cognition in Neurological Disorders. Nutrients 13, 2099. doi: 10.3390/nu13062099

Coy, S., Rashid, R., Stemmer-Rachamimov, A., and Santagata, S. (2020). An Update on the CNS Manifestations of Neurofibromatosis Type 2. Acta Neuropathol. 139, 643–665. doi: 10.1007/s00401-019-02029-5

Cryan, J. F., O’Riordan, K. J., Cowan, C. S. M., Sandhu, K. V., Bastiaanassen, T. F. S., Boehme, M., et al. (2019). The Microbiota-Gut-Brain Axis. Physiol. Rev. 99, 1877–2013. doi: 10.1152/physrev.00018.2018

SUPPLEMENTARY FIGURE 1 | Heat map of the relative abundances of the top 60 dominant taxa at genus level in all samples. The genera were displayed from higher abundance (in red) to lower abundance (in blue).
Cryan, J. F., O’Riordan, K. J., Sandhu, K., Peterson, V., and Dinan, T. G. (2020). The Gut Microbiome in Neurological Disorders. Lancer Neurol. 19, 179–194. doi: 10.1016/s1474-4422(19)30536-4

D’Alessandro, G., Antonangeli, F., Marrocco, F., Porzia, A., Lauro, C., Santoni, A., et al. (2020). Gut Microbiota Alterations Affect Glioma Growth and Intraneural Immune Cells Involved in Tumor Immunosurveillance in Mice. Eur. J. Immunol. 50, 705–711. doi: 10.1002/eji.201948354

Daille, B., Van Oudenhove, L., Vervliet, B., and Verbeke, K. (2019). The role of short-chain fatty acids in microbiota-gut-brain communication. Nat. Rev. Gastroenterol. Hepatol. 16, 461–478. doi: 10.1038/s41575-019-0157-3

Dehaghi, M., Kazemi Shariat Panahi, H., Heng, B., and Guillemin, G. J. (2020). The Gut Microbiota, Kynurenine Pathway, and Immune System Interaction in the Development of Brain Cancer. Front. Cell Dev. Biol. 8. doi: 10.3389/fcell.2020.562812

Di Modica, M., Gargari, G., Regondi, V., Bonizzi, A., Arioli, S., Belmonte, B., et al. (2021). Gut Microbiota Condition the Therapeutic Efficacy of Trastuzumab in HER2-Positive Breast Cancer. Cancer Res. 81, 2195–2206. doi: 10.1158/0008-5472.can-20-16507

El-Ansary, A. K., Ben Bacha, A., and Kotb, M. (2012). Etiology of Autistic Features: The Persisting Neurotoxic Effects of Propionic Acid. J. Neuroinflamm. 9, 74. doi: 10.1186/1742-2094-9-74

Fan, Y., and Pedersen, O. (2021). Gut Microbiota in Human Metabolic Health and Disease. Nat. Rev. Microbiol. 19, 55–71. doi: 10.1038/s41579-020-0433-9

Feng, Q., Chen D. B., and Wang, Y. D. (2018). Gut Microbiota: An Integral Mediator in Health and Disease. Front. Microbiol. 9. doi: 10.3389/fmicb.2018.00151

Fernandes, M. F., de Oliveira, S., Portovedo, M., Rodrigues P. B., and Vinolo, M. A. R. (2020). Effect of Short Chain Fatty Acids on Age-Related Disorders. Adv. Exp. Med. Biol. 1260, 85–105. doi: 10.1007/978-3-030-24667-5_4

Guo, W. J., Yuan, J. J., Li, H. M., Huang, Y., Chen, C. L., et al. (2018). Proteobacteria Community Compositions Correlate With Bronchiectasis Severity. Int. J. Tuberc. Lung Dis. 22, 1095–1105. doi: 10.5588/ijtld.18.0037

Gusyatiner, O., and Hegi, M. E. (2018). Gliala Epigenetics: From Subclassification to Novel Treatment Options. Semin. Cancer Biol. 51, 50–58. doi: 10.1016/j.semcancer.2017.11.010

Jena, P. K., Sheng, L., Nagar, N., Wu, C., Barile, D., Mills, D. A., et al. (2018). Symbiotics Bifidobacterium Infantis and Milk Oligosaccharides are Effective in Reversing Cancer-Prone Nonalcoholic Steatohepatitis Using Western Diet-Fed FXR Knockout Mouse Models. J. Nutr. Biochem. 57, 246–254. doi: 10.1016/j.jnutbio.2018.04.007

Jiang, S. H., Hu, L. P., Wang, X., Li, J., and Zhang, Z. G. (2020). Neurotransmitters: The Influence of the Gut Microbiome, Diet, and Environment on Risk of Colorectal Cancer. Gastroenterology 158, 322–340. doi: 10.1053/j.gastro.2019.06.048

Taverniti, V., Cesari, V., Gargari, G., Rossi, U., Biddau, C., Lecchi, C., et al. (2021). Probiotics Modulate Mouse Gut Microbiota and Influence Intestinal Immune and Serotonergic Gene Expression in a Site-Specific Fashion. Front. Microbiol. 12. doi: 10.3389/fmicb.2021.706135

Tran, S. M., and Mohajeri, M. H. (2021). The Role of Gut Bacterial Metabolites in Brain Development, Aging and Disease. Nutrients 13, 732. doi: 10.3390/nu13030732

Vaz-Moreira, I., Nunes O., C., and Manaa, M. C. (2017). Ubiquitous and Persistent Proteobacteria and Other Gram-Negative Bacteria in Drinking Water. Sci. Total Environ. 586, 1141–1149. doi: 10.1016/j.scitotenv.2017.02.104

Vivarelli, S., Saleni, R., Candido, S., Falzone, L., Santagati, M., Stefani, S., et al. (2019). Gut Microbiota and Cancer: From Pathogenesis to Therapy. Cancers (Basel) 11, 38. doi: 10.3390/cancers11010038

Wang, X., Sun, G., Feng, T., Zhang, J., Huang, X., Wang, T., et al. (2019). Sodium Oligomannate Therapeutically Remodels Gut Microbiota and Suppresses Gut Bacterial Amino Acids-Structured Neuroinflammation to Inhibit Alzheimer’s Disease Progression. Cell Res. 29, 787–803. doi: 10.1038/s41422-019-0216-x

Wexler, H. M. (2007). Bacteroides: The Good, the Bad, and the Nitty-Gritty. Clin. Microbiol. Rev. 20, 593–621. doi: 10.1128/cmrr.00008-07

Wong, S. H., and Yu, J. (2019). Gut Microbiota in Colorectal Cancer: Mechanisms of Action and Clinical Applications. Nat. Rev. Gastroenterol. Hepatol. 16, 690–704. doi: 10.1038/s41575-019-0209-8

Yoo, J. Y., Groer, M., Dutra, S. V. O., Sarkar, A., and McSkimming, D. I. (2020). Gut Microbiota and Immune System Interactions. Microorganisms 8, 1587. doi: 10.3390/microorganisms8101587
Zmora, N., Suez, J., and Elinav, E. (2019). You are What You Eat: Diet, Health and the Gut Microbiota. *Nat. Rev. Gastroenterol. Hepatol.* 16, 35–56. doi: 10.1038/s41575-018-0061-2

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