5-Fluorouracil induced dysregulation of the microbiome-gut-brain axis manifesting as depressive like behaviors in rats

Fan Zhang a,b,1, Haitao Chen a,1, Ruixin Zhang b, Yu Liu c, Ning Kong a, Yong Guo d,⁎, Maosheng Xu b,⁎

a The First Clinical College of Zhejiang Chinese Medical University, Hangzhou, Zhejiang 310053, China
b Department of Radiology, The First Affiliated Hospital of Zhejiang Chinese Medical University, Hangzhou, Zhejiang 310003, China
c College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang 310000, China
d Department of Oncology, First Affiliated Hospital of Zhejiang Traditional Medical University, Hangzhou, Zhejiang 310003, China

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ABSTRACT

Disturbances of the gut microbiome have been widely suggested to be associated with 5-fluorouracil (5-Fu) induced digestive pathologies. Furthermore, it has been elucidated that the gut microbiome may play a key role in the pathogenesis of depressive disorders via the microbiota-gut-brain axis. Despite the speculation, there exists no direct evidence proving the causality between disturbances in the gut microbiome induced by 5-Fu and depressive mood dysregulation. Herein, behavioral testing was used to evaluate depressive-like behaviors in 5-Fu treated rats. Subsequently, the gut microbiota and prefrontal cortex (PFC) metabolic were analyzed by 16S rRNA sequencing and 1H nuclear magnetic resonance (1H NMR). To clarify the association between the gut microbiota and their role on depressive-like behaviors caused by 5-Fu, a fecal microbiota transplantation (FMT) experiment was carried out. The results suggested that 5-Fu could significantly alter the diversity and abundance of the gut microbiome, and induce PFC metabolic disorders, as well as depressive behaviors in rats. Transplantation of fecal microbiota from healthy control into 5-Fu treated rats significantly alleviated the PFC metabolic disorder and depressive-like behaviors. In conclusion, this study demonstrated that the gut microbiome was actively involved in the occurrence of 5-Fu induced depressive-like behaviors, and manipulation of specific gut microbiome parameters may serve as a promising novel target for side effects of 5-Fu treatment.

1. Introduction

5 Fluorouracil (5-Fu) and its derivatives have been the first choice for chemotherapy in colorectal cancer (CRC) for many years [1,2]. However, chemotherapy functions as a double-edged sword. Damage to tumor cells must be balanced with damage to healthy tissues at the same time, not surprisingly mood disorders such as depression are a commonly reported and disruptive issue for cancer patients after chemotherapy [3]. These negative emotions may affect patients' compliance and affect the follow-up to treatment [4,5]. Depression is a complex, multifactorial disorder and has been associated with changes in several physiological pathways, including adipose-derived hormones [6], inflammatory cytokines [7], the hypothalamic-pituitary-adrenal axis [8], and oxidative stress pathways [9].

Although depression is primarily a psychological condition, the overall physiology of depression is embedded in the central nervous system (CNS) [10]. The prefrontal cortex (PFC) plays a significant role in regulating emotion, memory, cognition, and learned responses [11,12]. There is an abundance of neuroscientific evidence from functional imaging, lesion studies, and brain stimulation studies that have implicated the role of the PFC in depression [13]. For instance, depression patients show gray matter density abnormalities in the right dorsolateral prefrontal cortex (DLPFC) [14], and dysconnectivity between the PFC and other regions of the brain [15]. A variety of preclinical magnetic resonance spectroscopy (MRS) studies has reported associations among alterations in glutamate, GABA, creatine and inositol levels in the PFC of depressed patients [16,17]. These results strongly suggest that a relationship exists between the metabolism of the PFC and depression.

The gut microbiome is composed of a wide variety of microbial species [18], with evidence suggesting that disturbances in the gut microbiome are strongly associated with pathologies, such as depression [19,20]. The relationship between the microbiome and psychiatric symptoms appears to be bidirectional [21]. Thus, the microbiota–gut–brain axis may play a key role
in maintaining overall health and mental health disorders [22,23]. 5-Fu can induce intestinal mucosal damage and cause gastrointestinal symptoms such as vomiting and diarrhea, which are also associated with gut microbiota disorders [24]. Specifically, 5-Fu can lead to a lower relative abundance of Firmicutes, and an increase in Bacteroidetes, Actinobacteria and Verrucomicrobia [20,24,25]. Interestingly a similar phenomenon has been observed by Jiang et al. [20] on Major Depressive Disorder (MDD), which revealed a high level of Bacteroidetes, Proteobacteria, and Actinobacteria, as well as low levels of Firmicutes compared to the healthy controls in depressed individuals. Yet, few reports have investigated whether 5-Fu can cause depressive behaviors, and whether the occurrence of such behaviors is related to the imbalance of gut microbiome.

To solve these issues, we initially performed behavioral experiments to determine whether the rats developed depression-like behaviors after 4 days 5-Fu treatment, followed by applying a 16S rRNA gene sequencing to evaluate the specific alterations in the gut microbiome. Then, 1H nuclear magnetic resonance (NMR) spectroscopy-based metabolic phenotyping was used to investigate the changes of PFC metabolites affected by 5-Fu. Furthermore, to assess the correlations between alterations of gut microbiome, depressive-like behaviors and disorders of PFC metabolites, fecal microbiome transplantation (FMT) was performed from healthy control rats to 5-Fu treatment rats.

2. Materials and methods

2.1. Animals

Male, 5 week old Sprague-Dawley (SD) rats were purchased from Shanghai SIPPR-BK Laboratory Animal Co. Ltd. All rats were randomly assigned in five per cage and fed the same autoclaved chow and water maintained on a 12:12-h light-dark cycle environment (temperature of 21 ± 1 °C, humidity of 55 ± 10%, low noise). Interventions began after one week of adaptive feeding. Weight and food intake were recorded daily for 4 days. This experiment was supervised and approved by Experimental Animal Ethical Committee of the Zhejiang Chinese Medical University (Approved No. of ethic committee: ZSLL-2018-014).

20 SD rats were randomly divided into 2 groups: Control group (n = 10), 5-Fu treatment group (n = 10). 5-Fu group rats were given an intraperitoneal injection of 40 mg/kg 5-Fu (Shanghai Xudong Haipu pharmaceutical Co., Ltd., China) once a day for 4 days, while control group rats received the same amount of saline. During the experiment, all rat feces were collected daily from each rat and refrigerated at −80 °C for fecal microbiome analysis and transplantation.

2.2. Behavioral testing

2.2.1. Open-field test (OFT)

All rats were randomly selected and individually tested in an open-field apparatus composed of 16 consisting of a black square base (100 × 100 cm²) with black walls (40 cm in height). Rats were placed in one corner of the apparatus for 1 min to acclimate. All spontaneous activities were recorded (5 min) and calculated by an automated motor activity recording tracker (SMART 3.0, Panlab, Barcelona, Spain). Considering the total distance of movement as an indicator of locomotor activity, the proportion of the distance spent in the center of the board (25% of the inner surface area) was used as an indicator of anxiety-like behavior. The apparatus was cleaned before and after each experiment.

2.2.2. Tail suspension test (TST)

The rats were randomly selected and individually suspended by their tails using adhesive tape (2 cm from the tip of tail). The test took place between 8 a.m. and 12:30 a.m. and lasted for 6 min, with the last 5 min scored for immobility. Rats that climbed on their tails were excluded from further testing. Animals were considered to be immobile when they exhibited no body movement and hung passively.

2.2.3. Sucrose preference test (SPT)

Before the test, all the rats were kept in a single cage and given 1% sucrose solution for 24 h. Afterward, a bottle of white water and 1% sucrose solution were given for 24 h and the position of the two bottles were varied every 6 h. On the experimental day, water and food were removed from the cage for 6 h. All rats were then given the same pre-weighed bottles containing 1% sucrose solution and water. All fluid consumption was recorded by weighing the two bottles before and 1 h after the start of the test. After 12 h, the bottles were weighed and the sucrose preference index was defined as (sucrose solution consumption/total consumption) × 100%.

2.3. 16S rRNA Miseq sequencing and bioinformatic analysis

The total genomic DNA of the gut microbiome was extracted from 200 mg of fecal samples using the E.Z.N.A. *Stool DNA Kit (D4015, Omega, Inc., USA) following the manufacturer’s instructions. Then, the V3-V4 region of the bacterial 16S rRNA gene in each sample was amplified with primers 341F (5′-CCTACGGGNGGCWGCAG-3′) and 805R (5′-GACTACHVGGGTATCTAATCC-3′). The process of PCR amplification was conducted as described previously [26]. Subsequently, a mixture of PCR products were purified by AMPure XT beads (Beckman Coulter Genomics, Danvers, MA, USA) and quantified by Qubit (Invitrogen, USA). The 16S rRNA gene sequences were then analyzed using an Illumina NovaSeq platform. According to the truncated barcode and primer sequence of the samples, the paired-end reads were assigned to the samples. Paired-end reads were merged using Fast Length Adjustment of Short Reads (FLASH). According to qftrim (version 0.94), the data was filtered under filtering conditions to obtain high-quality, clean labels. Then, the chimera sequences were filtered using Vsearch software (version 2.3.4). These sequences were clustered to operational taxonomic units (OTUs) with a similarity greater than 97% and were assigned to the same OTUs. Alpha diversity and beta diversity were calculated by QIIME2. The same number of sequences were extracted randomly by reducing the number of sequences to the minimum of samples. The relative abundance (X bacteria count/total count) was used for bacteria taxonomy. Alpha diversity and Beta diversity were analyzed by the QIIME2 process. The sequence alignment of the species annotation was performed by Blast, and the alignment database used was the SILVA and NT-16S.

2.4. Brain tissue preparation for 1H NMR analysis

At the end of the experiment (9 days), rats were anesthetized by 10% chloral (0.4 ml/g). The whole brain was removed, snap-frozen in liquid nitrogen, and stored at −80 °C for metabolomics analysis. 100 mg of PFC tissue were homogenized in 800ul of a 4.0:8.5 (v/v) MeOH/H₂O solution for polar metabolite extraction. Tissue was homogenized using Qiagen a TissueLyser II (Retsch, Germany) at a frequency of 1/30 for 6 min followed by centrifugation at 13,000 RCF at 4 °C for 10 min. Then, 500 μl of supernatant was collected. Methanol was removed by a vacuum centrifugal concentrator (labconco, USA). The aqueous phase was reconstituted in 600 μl of 99.5% D2O phosphate buffer pH 7.4 (containing 0.05% 3-trimethylsilyl-propionate-d4; SIGMA, USA) and transferred to 5-mm NMR tubes for 1H NMR analysis.

2.5. 1H NMR spectroscopy analysis and data processing

All 1H NMR spectra were recorded by Bruker 600 MHz AVANCE III spectrometer equipped with a 5 mm-BBFO probe at 25 °C. Shimming and proton pulse calibration were performed automatically for each sample before data acquisition. 1H NMR spectra were received using NOESYPR 1D pulse sequence with water suppression. The data was processed using Bruker Topspin 3.2.

Free induction decays (FIDs) from 1H NMR of the PFC were multiplied by a 0.3 Hz exponential line broadening prior to Fourier
Transformation. All obtained NMR spectra were manually phased, baseline corrected and referenced to TSP (δ = 0.0) within MestReNova 12 (Mestrelab Research SL, Spain). The integral region of the spectrum was set between 0.0 and 9.0 ppm, with a spectral region of 4.5–5.0 ppm to eliminate the effects of imperfect water suppression. Due to the deviation of metabolite concentration in the gastrocnemius tissue of each rat, each bucket was internally normalized to the total sum of the spectral integrals prior to pattern recognition analysis. The characteristic peaks of all brain metabolites were determined based on related literature [27–29] and the Biological Magnetic Resonance Bank (http://www.bmrb.wisc.edu/metabolomics) and Human Metabolome Database (http://www.hmdb.ca/).

2.6. Fecal microbiota transplantation

For FMT, 16 rats were randomly divided into two groups: 5-Fu group (n = 8) and 5-Fu + FMT group (n = 8). Both groups received an intraperitoneal injection of 40 mg/kg 5-Fu once a day for 4 days. Fecal materials from normal rats were homogenized, dissolved in PBS and settled by gravity for 10 min before transplantation. Rats of the 5-Fu + FMT group were fed with the fecal suspension via oral gavage once a day for 4 days, while 5-Fu group rats received the same amount of PBS. For the reason that pre-treatment with antibiotics provided minimal impact on the efficacy of fecal transplants [30,31], rats of this test were not pretreated with antibiotics.

2.7. Statistical analysis

The metabolomic datasets were first normalized to a constant sum and scaled. Then, principal components analysis (PCA) and partial least-squares discriminant analysis (PLS-DA) were performed using SIMCA software 13.0 (Umetrics AB, Umea, Sweden). Metabolites between two groups were analyzed by an unpaired Student’s t-test using GraphPad Prism 6.01. All values were expressed as mean ± S.D. and P < 0.05 was considered as statistically significant. The differential metabolites were filtered by variable influence on projection (VIP) selection according to the PLS-DA with the filtering conditions of VIP > 1 at multivariate statistical analysis and P < 0.05 at univariate statistics were adopted to identify the differential metabolites. 6 differential metabolites were then selected by the aforementioned criteria, including GABA, glutamine, glycine, isobutyrate, glutamate, phosphocholine, inosine, methanol, acetate, choline, fumarate, inositol, and alanine in 5-Fu treatment rats which significantly lower than those in control rats (P < 0.05). Additionally, nicotine acid in 5-Fu treatment rats increased significantly relative to that of control rats (Supplemental Table 1). Furthermore, the criteria of both VIP > 1 at multivariate statistical analysis and P < 0.05 at univariate statistics were adopted to identify the differential metabolites. 6 differential metabolites were then selected by the aforementioned criteria, including GABA, glutamine, glycine, isobutyrate, acetate and nicotine acid (Fig. 3c). On the basis of the differential metabolites, relevant metabolic pathways were identified by KEGG and HMDB databases. Those pathways with an impact value > 0.1, P < 0.05 and false discovery rate (FDR) q value < 0.1 were considered as the most relevant pathways involved in depression-like behaviors of rats induced by 5-Fu. Accordingly, two metabolic pathways, i.e. (1) Glyoxylate and dicarboxylate metabolism, (2) Alanine, aspartate and glutamate metabolism, were recognized as the most influenced metabolic pathways associated with 5-Fu treatment (Fig. 3d). More information about 5-Fu Caused Changes in PFC Metabolism could be found in Supplementary data (Tables S1, Fig. S1).

3. Results

3.1. 5-Fu treatment induced depressive-like behaviors

The body weight and food intake of 5-Fu treatment group decreased significantly compared with the control group (P < 0.001, Fig. 1a, b). From the OFT, no difference in total motion distance and distance in center was found between the 5-Fu group and the control group (Fig. 1c, d). In the TST and SPT, 5-Fu treatment rats displayed significantly increased sedentary time (Fig. 1e) and a notable decrease in depressive-like behaviors treated by 5-Fu (Fig. 1f).

3.2. Fu altered gut microbiome diversity and community composition

A lower Shannon diversity index and Simpson Evenness was observed in the 5-Fu group compared to the control group (Wilcoxon’s rank-sum test; p < 0.05; Fig. 2a, b). Chao1 and Observed Specie were both significantly different between the 5-Fu and control groups (Wilcoxon’s rank-sum test; p < 0.05; Fig. 2c, d). The principal coordinate analysis (PCoA) scatter plot demonstrated that the composition of the gut microbiome was significantly altered from the 5-Fu group to the control group at the genera level (Fig. 2e). Moreover, 5-Fu treatment decreased the relative abundance of Firmicutes and Bacteroidetes. However, 5-Fu increased the abundance of Proteobacteria and Verrucomicrobia at the phylum level (Fig. 2f). In addition, based on observed microbial differences at the phylum level in the control group and 5-Fu group, the relative abundance of the top 30 genera was presented with a heatmap (Fig. 2g). Compared with the control group, Lactobacillus, Muribaculaceae, f_Lachnospiraceae, f_Ruminococcaceae, Eubacterium, Ruminiclostridium, p_Firmicutes, Romboutsia, Clostridium, Dunca niella, Ruminococcus, Clostridiales, Oscillibacter, Intestinimonas and Peptococcaceae were reduced, while Akkermansia, Escherichia/Shigella, Blautia, Bifidobacterium, Enterococcus, Bacteroides, Parasutterella, Dubosiella, Lachnoclostridium Allobaculum and Erysipelotrichaceae had greater abundance in the 5-Fu treatment group. These results indicated that 5-Fu treatment reduced the diversity and changed the composition of the bacterial community.

3.3. 5-Fu caused changes in PFC metabolism

The neurochemistry in brain tissue of PFC was examined by 1H NMR spectroscopy. A total of 26 neural metabolites were examined. The unsupervised PLS-DA score plots showed that the 5-Fu treatment and control group were clearly distinct from one another (Fig. 3a). Such alterations indicated different metabolic patterns between the two groups. The validation plot based on 1H NMR spectra of both groups illustrated that the PLS-DA model was robust and credible (R2X, R2Y and Q2 values of PLS-DA models are 0.825, 0.973, 0.883 respectively. Fig. 3b).

There were 13 chemicals including γ-Aminobutyric acid (GABA), glutamine, glycine, isobutyrate, glutamate, phosphocholine, inosine, methanol, acetate, choline, fumarate, inositol, and alanine in 5-Fu treatment rats which significantly lower than those in control rats (P < 0.05). Additionally, nicotine acid in 5-Fu treatment rats increased significantly relative to that of control rats (Supplemental Table 1). Furthermore, the criteria of both VIP > 1 at multivariate statistical analysis and P < 0.05 at univariate statistics were adopted to identify the differential metabolites. 6 differential metabolites were then selected by the aforementioned criteria, including GABA, glutamine, glycine, isobutyrate, acetate and nicotine acid (Fig. 3c). On the basis of the differential metabolites, relevant metabolic pathways were identified by KEGG and HMDB databases. Those pathways with an impact value > 0.1, P < 0.05 and false discovery rate (FDR) q value < 0.1 were considered as the most relevant pathways involved in depression-like behaviors of rats induced by 5-Fu. Accordingly, two metabolic pathways, i.e. (1) Glyoxylate and dicarboxylate metabolism, (2) Alanine, aspartate and glutamate metabolism, were recognized as the most influenced metabolic pathways associated with 5-Fu treatment (Fig. 3d). More information about 5-Fu Caused Changes in PFC Metabolism could be found in Supplementary data (Tables S1, Fig. S1).

3.4. Disturbed gut microbiota was involved in disorder of PFC metabolites and depressive-like behaviors treated by 5-Fu

As shown in Fig. 4a and b, FMT significantly reverted body weight loss in 5-Fu treatment rats (P < 0.05). Furthermore, FMT significantly reduced sedentary time in TST (P < 0.01), while increasing the sugar preference rate in SPT (P < 0.05, Fig. 4c, d). These results implicated that disruptions in mood caused by 5-Fu treatment were, in part, due to simultaneous disruptions in the gut microbiome.

After healthy rat feces were transplanted to the 5-Fu treatment group, the unsupervised PLS-DA score plots showed that the 5-Fu treatment and control group separated clearly from each other (Fig. 5a).
Fig. 1. Effect of 5-Fu treatment on body weight, food-intake and mood-related behavior. (a, b) Body weight, food-intake of control group rats and 5-Fu treatment rats (n = 10/group). (c, d) Open-field test (OFT): the total motion distance and proportion of central motion distance showed no difference between control and 5-Fu treatment rats (n = 10/group). (e) Tail suspension test (TST): 5-Fu treatment group showed a significantly increased immobility time than the control group (n = 10/group). (f) Sucrose preference test (SPT): 5-Fu treatment significantly decreased the sucrose preference index than control group (n = 10/group). Data was shown as mean ± standard errors of the mean. ***P < 0.001 by t-test.
Furthermore, the validation plot based on the $^1$H NMR spectra of both groups illustrated that the PLS-DA model was robust and credible (R$^2_X$, R$^2_Y$ and Q$^2$ values of PLS-DA models are 0.718, 0.962, 0.889 respectively. Fig. 5b). The enhanced metabolism in the PFC induced by 5-Fu were all downregulated, such as nicotinic acid, N-acetylaspartate, fumarate, creatine, methionine, glutamine, glutamate, glycine, 3-
hydroxyphenylacetate, GABA, aspartate, succinate, acetate, alanine, isobutyrate, valine and isoleucine (Fig. 5c). It is suggested that 5-Fu can affect the CNS through microbiota-gut-brain axis.

4. Discussion

The present study has shown that 5-Fu can induce depressive-like behaviors in rats, as well as disorders of gut microbiota and PFC metabolism. Through FMT it was suggested that these depressive-like behaviors and the result of metabolic changes in the PFC and associated with disorders of the gut microbiota.

To determine whether 5-Fu can cause depressive-like behaviors in rats, three preclinical models (OFT, SPT and TST) were used for assessing depressive-like behaviors. Compared with the control group, 5-Fu treated subjects exhibited significant decreases weight, food intake and sucrose preference in SPT, as well as increases in sedentary time in TST. However, no significant difference was observed between the two groups in OFT. OFT is widely used for assessment of locomotor activity and anxiety-like behavior. Anxiety is a common symptom of depression, and almost two-thirds of MDD patients also suffer from clinical anxiety [32].

Combined with data on PFC metabolism in rats, the concentration of GABA in 5-Fu group was significantly higher than the control group. As the most abundant inhibitory neurotransmitter in the central nervous system [33], GABA plays an important role in the central modulation of anxiety and stress responses [34,35]. He et al. [36] reported that oral administration of GABA could increase the distance and time in OFT of an emotional stress rat model. In human studies, Abdou et al. [37] found that oral intake of GABA markedly increased alpha waves and decreased beta waves in the brain. Finally, they concluded that GABA could induce a calming effect and diminish anxiety. These findings consistently demonstrate that 5-Fu can induce depressive-like behaviors in rats, which are manifested as anhedonia and behavioral despair, rather than anxiety-like behavior.

Disruptions of the gut microbiota, both in diversity and abundance, have been found to play an important role in the development of depression [19,38]. In our study, the gut microbiome displayed significant taxonomical differences between the 5-Fu treated and control group. At the phylum level, Proteobacteria Verrucomicrobia were significantly increased in the 5-Fu treatment group, whereas the content of Firmicutes and Bacteroidetes was markedly lower than control group. Numerous studies have showed that an increase in the relative abundance of Proteobacteria in the gut could reflect dysbiosis or an unstable gut microbial community structure despite their low abundance [39,40]. In addition, the expansion of gut Proteobacteria reflects an energy imbalance of the host and a positive correlation between the susceptibility to colitis [40]. Previous studies regarding 5-Fu showed a relative decrease in abundance in Firmicutes [25,41]. However, the relationship between the abundance of Firmicutes and mood dysregulation is mostly unclear [20]. At the genus level, we observed that 5-Fu significantly decreased Lactobacillus and Eubacterium, and increased Akkermansia in rat feces. The relative amount of Eubacterium is responsible for mood swings. It has been reported that an increased Montgomery-Asberg Depression Rating Scale score in depressed patients was associated with a decrease in eubacteria [42]. Moreover, Eubacterium is a producer of
short-chain fatty acids (SCFA) [43], and SCFAs play a protective role against inflammation in the gut [44]. Probiotic bacteria are defined as live microorganisms that can inhabit the gut and contribute to the health of the host [45], Lactobacillus and Akkermansia are such examples of probiotic bacteria. A growing body of evidence suggests that oral administration of probiotic containing Lactobacillus can lead to antidepressant-like effects in rodents [46,47]. Akkermansia has been demonstrated to have a positive effect against obesity, insulin resistance, and diabetes [48,49], as well as a beneficial role in attenuated inflammation and rebalancing of the gut microbiota [50]. However, both this study along with Hamouda et al. [25] have observed that the abundance of Akkermansia was significantly increased after 5-Fu treatment. These results suggested that in certain situations, a relative higher abundance of certain probiotics may reflect signal changes of gut biology.
the environment, and the accumulation of probiotics in the gut may not always reflect an overall state of health.

Brain imaging studies have shown that alterations in PFC structure and function have been associated with depression, as such alterations may be related to dysregulation in the glutamatergic system in the central nervous system [51–53]. A recent meta-analysis on 17 reports [54] and MRS studies have showed a reduction in glutamate and glutamine in the PFC of depressed patients and rat models [55,56]. Yet, the relationship between 5-Fu and depression in rats was not elucidated prior to this study. We speculate that these differences may be related to the type and duration of stress. Dayas et al. [57] proposed that stressors should be divided into at least two broad categories, “physical” and “psychological”. While the side effects of 5-Fu can be broken into physical and psychological components, the short time span of this experiment better lends itself to creating a condition of acute stress. This condition likely resulted in increased glutamate metabolism in the PFC secondary to chemical stress induced by 5-Fu [58,59]. Glutamate is the most abundant amino acid in the brain and the primary excitatory neurotransmitter in the CNS. Glutamate drives about 85% of synaptic neurotransmission and plays important roles in neuronal growth, brain development and maturation, synaptic plasticity in health and disease [60], but high concentrations of glutamate in the synaptic cleft can be toxic and may lead to over-activation of its receptors and atrophy of neurons [61]. This phenomenon is generally termed as glutamate excitotoxicity [62]. Glutamate excitotoxicity mediated by abnormal activation of glutamate receptors, notably of N-methyl-D-aspartate (NMDA) receptors, has been related to the many nervous system and psychiatric disorders, including stroke [63], neurodegenerative disorders [64] and depression [65]. Taken together, we believe that 5-Fu treatment can contribute to acute depression due to the glutamate-mediated excitotoxicity hypothesis as a result of disruptions in the gut microbiome.

Previous works indicate that the gut microbiome plays an important role in the pathophysiology of depression [66,67], in order to determine whether the gut microbiome plays a causative role in depressive-like behavior caused by 5-Fu, a FMT experiment was performed. It was found that transplantation of healthy rat feces into 5-Fu-treated subjects via oral administration was able to reverse depressive-like behaviors induced by treatment and normalize the metabolism of the PFC. To our surprise, FMT was able to reduce almost all amino acid imbalances induced by 5-Fu in the peripheral and central nervous systems [38,68]. These findings provide evidence that 5-Fu can induce disruptions in the gut microbiome that lead to depressive-like behavior.

In summary, the results presented herein suggest that treatment with 5-Fu is directly responsible for alterations in the microbiome-gut-brain axis. Due to a strong relationship between the natural gut flora and alterations in PFC metabolic activity, it can be suggested that 5-Fu can induce an increased susceptibility to depressive-like symptoms following treatment. For cancer patients undergoing 5-Fu chemotherapy, the introduction of mood disorders can have profound implications on overall treatment prognosis and the rate of follow up. Therefore, methods capable of mitigating this dysregulation may serve as promising additions for patients undergoing 5-Fu therapy to improve the overall quality of life.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

Experiments were conceived and designed by MSX and QHY. Animal experiments were performed by FZ and HTC. Sample collection and 1H NMR metabolomic analysis were primarily performed by FZ with contribution from HTC, RXZ, YL, NK. FZ and HTC wrote manuscript. All authors read, reviewed, edited and approved the manuscript.

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