BMC Cancer

RESEARCH Open Access

Multiple primary malignancies and gut microbiome

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Abstract

Background Multiple primary malignancies (MPM) are two or more independent primary malignancies. Recently, the relationship between microbiome and various tumors has been gradually focused on.

Objective To describe the relationship between MPM patients (MPMs) and gut microbiome.

Methods A total of 27 MPMs, 30 colorectal cancer patients (CRCs), and 30 healthy individuals were included to obtain metagenomic sequencing data. The knowledge graphs of gut bacteria and enteroviruses were plotted based on metagenomics. Wilcoxon rank-sum test was used to screen the characteristic gut microbiome.

Results The knowledge graph of gut microbiome in MPM patients was plotted. A total of 26 different gut bacteria, including *Dialister*, *Fecalibacterium* and *Mediterraneibacter*, were found between MPMs and healthy individuals. Twenty gut bacteria, including *Parvimonas*, *Dialister* and *Mediterraneibacter*, were more abundant in MPM complicated by CRC compared with CRCs. Twenty-one different enterovirus, including *Triavirus*, *Punavirus* and *Lilyvirus*, were screened between MPMs and healthy individuals. *Triavirus*, *Punavirus* and *Lilyvirus* were less abundant in MPM than healthy individuals. The abundance of *Triavirus*, *Punavirus* and *Lilyvirus* in CRC patients were also lower than MPM complicated by CRC patients.

Conclusion The knowledge graph of gut microbiome in MPM patients was plotted. It may provide basic data support for future research of MPM.

Highlights

- The knowledge graph of gut microbiome in MPM patients was plotted;
- There was a bigger number of gut microbes species(bacteria and virus) in MPM complicated by CRC patients compared with CRCs;
- *Dialister*, *Mediterraneibacter*, *Lilyvirus*, *Punavirus*, and *Triavirus* can be used as potential predictive biological targets for MPM concurrent CRC.

Keywords Multiple primary malignancies, Gut Microbiome, Metagenomic sequencing, Colorectal cancer

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Introduction

Multiple primary malignancies (MPM) are two or more independent primary malignancies. Recent years, MPM have gradually increased in incidence. Patients with previously diagnosed tumors have about a 20% risk of developing a new primary tumor, and about 30% of tumor survivors over the age of 60 have more than one tumor diagnosed [1, 2]. According to a study of nearly 430,000 patients diagnosed with at least two cancers, the most common subsequent cancers were squamous cell skin cancer and breast cancer [3]. Aging and variable tumor treatment were key factor contributing to develope a new malignancy [4–6]. Moreover, in the process of tumor treatment, chemotherapy and radiotherapy [7] and other treatment methods have also been proved to be carcinogenic factors [8]. For example, in the process of the first treatment of tumor, both radiotherapy and chemotherapy have significantly increased the risk of developing leukemia [9, 10]. In short, these results suggest that the tumor patients have a higher probability of developing tumors. In short, these results suggest that the tumor patients have a higher risk for developing to MPM.

The incidence of tumors is related to environmental factors, genetic factors, dietary factors, etc. Among them, 80–90% of malignant tumors are caused by external environmental factors [11], suggesting that environmental factors play a more major role, including viruses, bacteria, parasites, radiation and many chemicals [12]. Genetic susceptibility is difficult to change within a single generation, but studies have found that microbes can cause DNA damage, which would make oncogenes or anti-oncogene mutate leading to cancer [13]. In addition, dietary habits and food components will cause the gradual disturbance of gut microbiome community [14], which will also promote tumor occurrence. With the deepening of research, many researchers have gradually realized that the changes of gut microecosystem are an important inducing factor for tumors [15–18]. Therefore, gut microbiome may provide a new perspective and theoretical basis for the pathogenesis of MPM.

Recently, it has been reported that the polymorphism of the microbiome is included as one of the 14 biological characteristics of cancer [19]. It is well known that the colorectum is the main habitat organ of human microbiome due to its special location and function. Among the relationships between microbiome and various tumors, gut microbiome, including bacteria, viruses, archaic cells, and eukaryotes, is closely related to the occurrence and development of colorectal cancer(CRC) [20]. The incidence of CRC is as high as 19.9% in MPM [21], and the combination of colon cancer and rectal cancer is the most common MPM [22]. In addition to CRC, the gut microbiome has been linked to a variety of other tumors. For instance, *helicobacter pylori* can promote gastric cancer and gastric mucosa associated lymphoid tissue lymphoma (GALT) [23]. The gut microbiome may also increase the risk of breast cancer by metabolism, estrogen recycling, and immune pathways [24]. Gut bacteria may promote hepatocellular carcinoma (HCC) through proinflammatory microbiome-associated molecular patterns (MAMPs) and bacterial metabolites [25, 26]. Moreover, the changes in lung and gut microbiome function and MAMPS caused by immune processes, inflammation, and bacterial dysbiosis may be driving mechanisms of lung cancer [17]. However, most of the above studies focused on the relationship between gut bacteria and tumors, and there were few studies on viruses. Viruses are an important part of gut microbiome, and they are more diverse than bacteria. The imbalance of gut viruses can have a profound impact on human health. Therefore, it is more objective to study the gut microbiome related to MPM from multiple perspectives, such as bacteria and viruses.

In this study, metagenomic sequencing technology was used to annotate the microorganisms (including bacteria and viruses) in MPM patients. The analyses of gut microbial components, diversity analyses and difference analyses were further finished. The CRC patients and healthy individuals were used as controls to investigate the microbial signature of MPM. The knowledge graphs of gut microbiome in MPMs were plotted and the characteristic gut microbiome were screened to be intended as potential biological targets in MPM patients. It may provide a new perspective and method for the diagnosis and treatment of the disease in clinical practice, and also provide basic data support for subsequent research on MPM.

Materials and methods

Subjects

The subjects included 27 patients with multiple primary malignant tumors, 30 patients with CRC, and 30 healthy individuals admitted to Huzhou Central Hospital from January to December 2023. All the subjects provided signed informed consent under the guidance of the Ethics Committee of Huzhou Central Hospital. The patients' clinical protocol and informed consent were approved by the Ethics Committee of Huzhou Central Hospital (No. 202202005-02) and the Chinese Clinical Trial Registry (http://www.chictr.org.cn; ChiCTR2100050167). The specific clinical information of the volunteers involved in the data processing of this study came from the medical records management system of Huzhou Central Hospital.

To classify tumors as MPM, we used Warren and Gates criteria [27], (a) each tumor should be malignant, (b) each tumor should be histologically distinct, and (c) the possibility that one is a metastasis of the other must be ruled out. Two or more tumors found simultaneously in

the same patient or within 6 months of the initial diagnosis were considered synchronous tumors, while metachronous tumors were defined as tumors separated by more than 6 months [28]. Using the above criteria, The MPM patient samples selected for this study were all metachronous tumors.

Inclusion criteria for CRC patients, (a) patients with colorectal cancer confirmed by pathology; (b) Written informed consent was obtained from the applicant or his/her representative.

Exclusion criteria for CRC patients, (a) Patients with major diseases of the heart, brain, vascular, respiratory system, etc.; (b) Combined with chronic diseases such as hypertension and diabetes; (c) Combined with Crohn's disease, ulcerative colitis and other intestinal diseases; (d) Suffering from mental illness or cognitive and communication dysfunction.

Inclusion criteria of healthy individuals, (a) Colonoscopy results showed no intestinal lesions; (b) According to imaging, hematology and pathology indicators, the presence of existing cancer or previous cancer history was excluded; (c) Written informed consent was obtained from the patients or their proxies. With the informed consent of the patients, the basic information was obtained from the medical record management system of Huzhou Central Hospital (Table 1). $P > 0.05$, indicating that there was no significant difference in patient information.

Stool samples

Approximately 5–10 g of fecal samples were collected from patients who had not used laxatives or lubricants. The collection was performed using a fecal collector within 30 min after morning defecation and before breakfast. Each sample was carefully labeled and stored in an ultra-low temperature freezer at -80 °C for a maximum of one month.

Uniform quality control standards were applied to the fecal samples: A pre-test PCR amplification was conducted for quality assessment. The PCR primers used were 27 F (AGRGTTYGATYMTGGCTCAG) and 1492R (RGYTACCTTGTTACGACTT). If the purified PCR product yielded a weak band, the sample was excluded.

Metagenomic sequencing

Genomic DNA was fragmented into approximately 450 bp segments using the Covaris S220 focused-ultrasound instrument (Woburn, MA, USA). Sequencing libraries were prepared, and sequencing was performed using the Illumina HiSeq X platform in paired-end 150 bp (PE150) mode. The raw reads obtained were processed using Trimmomatic (http://www.usadellab.org/cms/uploads/supplementary) to remove low-quality sequences. The cleaned reads were then aligned to the human genome using the BWA-MEM algorithm (http://bio-bwa.sourceforge.net/bwa.shtml). Reads containing host genome contamination and low-quality sequences were filtered out, and the remaining high-quality data were retained for further analysis.The filtered data were compared against the UHGG (doi:https://doi.org/10.1038/s41587-020-0603-3) and MGV (doi:https://doi.org/10.1038/s41564-021-00928-6) databases to identify bacterial and viral species.

Data optimization and statistics

After stitching, quality control and joint removal, an optimized sequence was obtained. According to the optimized sequence, OTU clustering was performed to obtain an OTU abundance table.

OTU clustering analysis

OTU clustering was carried out using the Uparse method (version 7.1), with the sequence similarity threshold for OTUs set at 97%. Representative sequences for each OTU were obtained. To identify and remove chimeric

Table 1 The subjects of clinical basic information

		Health ($n=30$)	CRC ($n=30$)	MPM ($n=27$)	*P*
Age		67.267 ± 5.337	68.300 ± 4.872	69.407 ± 5.813	0.332
BMI		22.869 ± 2.965	23.143 ± 4.448	21.763 ± 3.416	0.364
Sex	Male	16	17	15	0.966
	Female	14	1613	12	
Diabetes	YES	34	3	7	0.1940.231
	NO	626	2627	20	
Hypertension	YES	511	910	8	0.9220.854
	NO	919	20	19	
TNM Stage	I	-	610	-	-
	II	-	25	-	
	III	-	109	-	
	IV	-	56	-	

sequences and PCR amplification errors, Uchime (version 4.2.40) was applied. The Usearch_global algorithm was used to align the optimized sequence data to the representative OTU sequences, generating a statistical table of OTU abundances for each sample, which was then utilized for subsequent bioinformatics analyses. The identified OTUs were screened and reviewed, focusing specifically on those within the bacterial domain. OTUs with abundances of 1 or 2, as well as OTUs annotated as chloroplasts or mitochondria, were excluded. OTU normalization was performed based on the sample with the lowest sequence count (35,000).

Bioinformatics analysis

(1) Microbial component analysis: The abundance of bacteria and viruses in the gut microbiome of different groups of individuals was described, and the main purpose was to show the microbial abundance of different populations. The analysis was completed by the "ggplot2" package of R language.
(2) Diversity analysis: The abundance and diversity of microbial community could be reflected by single sample diversity analysis (Alpha diversity). The Observed_species index was used to represent the number of OTUs detected. Chao index and Ace index were calculated using Mothur software (https://www.mothur.org/wiki/Download_mothur) to assess bacterial and virus abundance. Shannon index and Simpson index were calculated to evaluate microbial diversity. Pielou_J index is the ratio of Shannon index to the maximum Shannon index obtained from the community with the same species richness. The Graphpad software was used to draw the violin diagram and box plot.
(3) Difference analysis: Statistical analysis of abundance was performed to analyze statistically different bacteria at various levels (phylum, class, order, family, genus, and species levels). Wilcoxon rank-sum test was used to detect the characteristics of significant abundance difference and screen the groups with significant abundance difference. Finally, based on the statistical HVR, potential gut microbiome and viruses could differentiate between groups were identified, and available gut microbiome and viruses were screened for subsequent analysis. The gut microbiome and virus samples with a detection rate of less than 60% in the study samples were removed. This analysis can analyze the species or metabolites of the two groups of samples for significant differences. After difference analysis, False Discovery Rate (FDR) method was used to calibrate the p-value obtained and $p < 0.05$ indicates that there is a significant difference in the abundance of the genes in different groups.

Results

The knowledge graphs of MPMs

The 27 MPMs were divided into 20 groups according to tumor type and 30 CRCs and 30 healthy individuals were used as controls. The top 20 abundant gut bacteria, including *Veillonella*, *Streptococcus*, *Ruminococcus_E*, and the top 20 abundant enteroviruses, including *Uetakevirus*, *Triavirus*, *Teseptimavirus*, were selected to plot a component graph. The abundance of *Streptococcus* in CRCs complicated by rectum cancer (C + R) was significantly higher, the abundance of *Bacteroides* in CRCs complicated by thyroid cancer (C + T) was significantly higher, and the abundance of *Blautia* in CRCs complicated by Hepatocellular cancer (C + H) was significantly increased (Fig. 1A). In addition, the abundance of *Peduovirus* was significantly higher in patients with breast cancer and thymic carcinoma (B + Th) and the abundance of Lubbockvirus in CRCs complicated by thyroid cancer (C + T) was significantly increased (Fig. 1B).

The diversity of the gut microbiome in MPMs, CRC and health individuals

We selected 4 groups of MPM that were complicated by common cancers(MPM complicated by CRC, MPM complicated by lung cancer, MPM complicated by breast cancer and MPM complicated by stomach cancer) as main analysis objects. The CRC patients and healthy individuals served as control samples. Based on the top 30 gut bacteria with the highest abundance, the knowledge graphs of gut bacteria in the above 6 groups were plotted (Fig. 2A). The alpha diversity analysis were used to estimate their gut microbiome diversity. There were clear statistical differences in gut microbiome diversity between CRCs and MPM complicated by CRC ($p < 0.05$). As shown in Table S1, the Chao1 index and ACE index showed that MPMs complicated by CRC had a bigger number of species than CRCs. The Simpson index showed that the microbial diversity of MPMs complicated by CRC was higher compared with CRCs (Fig. 2B).

Moreover, based on the top 30 abundant enteroviruses, the knowledge graphs of enterovirus in the above 6 groups were plotted (Fig. 3A). As shown in Table S2, the Chao1 index and ACE index showed that MPMs complicated by CRC had higher species richness of enterovirus compared with CRCs ($p < 0.05$) (Fig. 3B).

The characteristic gut microbes(bacteria and virus) in MPMs

To further explore the valuable microbial targets of MPM patients, differential analysis of the gut microbiome data was performed. A total of 26 different gut

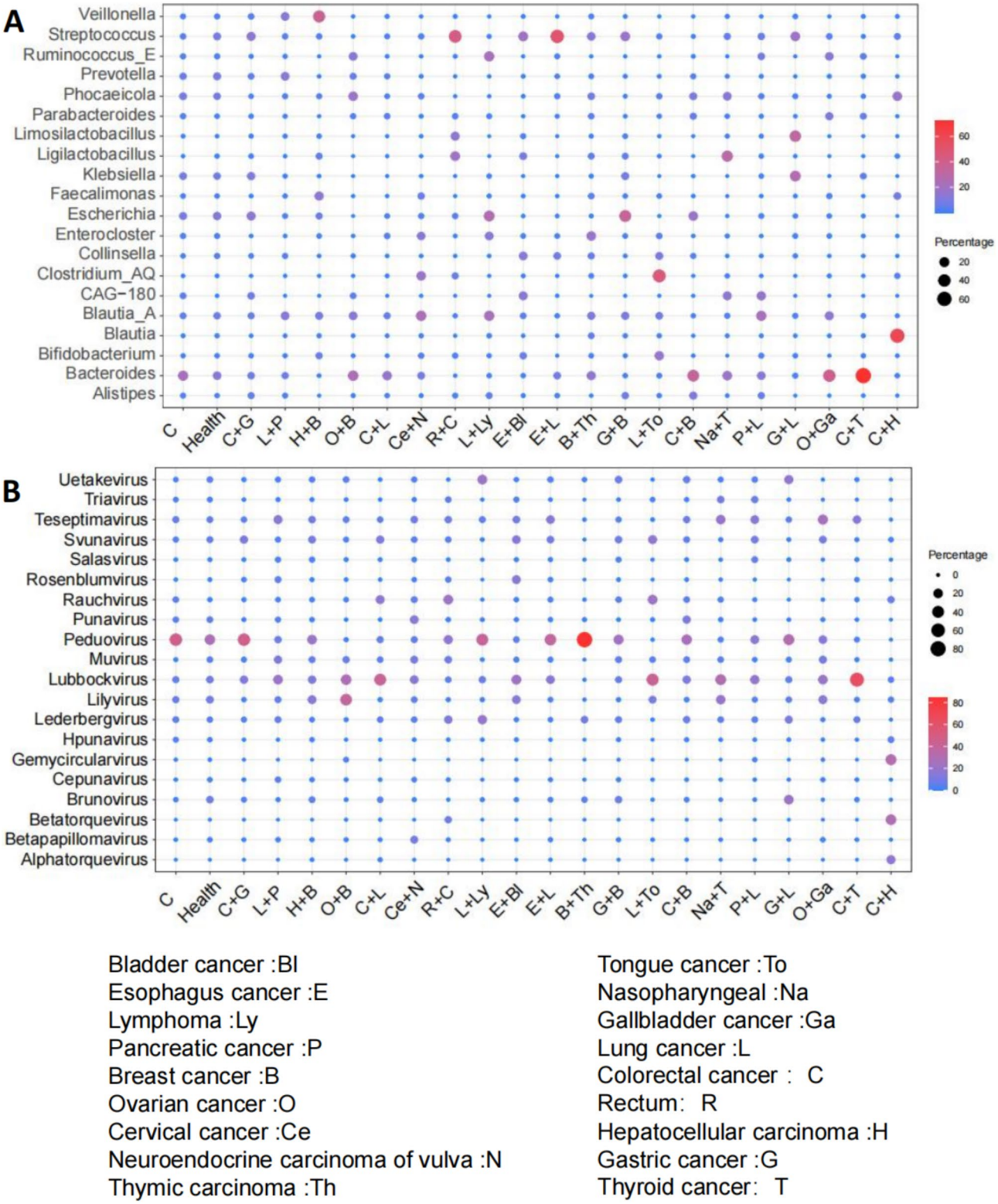

Fig. 1 Description of the abundance of gut microbiome. Bubble plots of the abundance of gut bacteria (Fig. 1**A**) and enteroviruses (Fig. 1**B**) in MPM, Health, CRC. The size of the bubbles directly reflects the abundance of gut bacteria or enteroviruses. The larger bubbles indicate a greater number or proportion of that gut bacteria or enterovirus in that sample

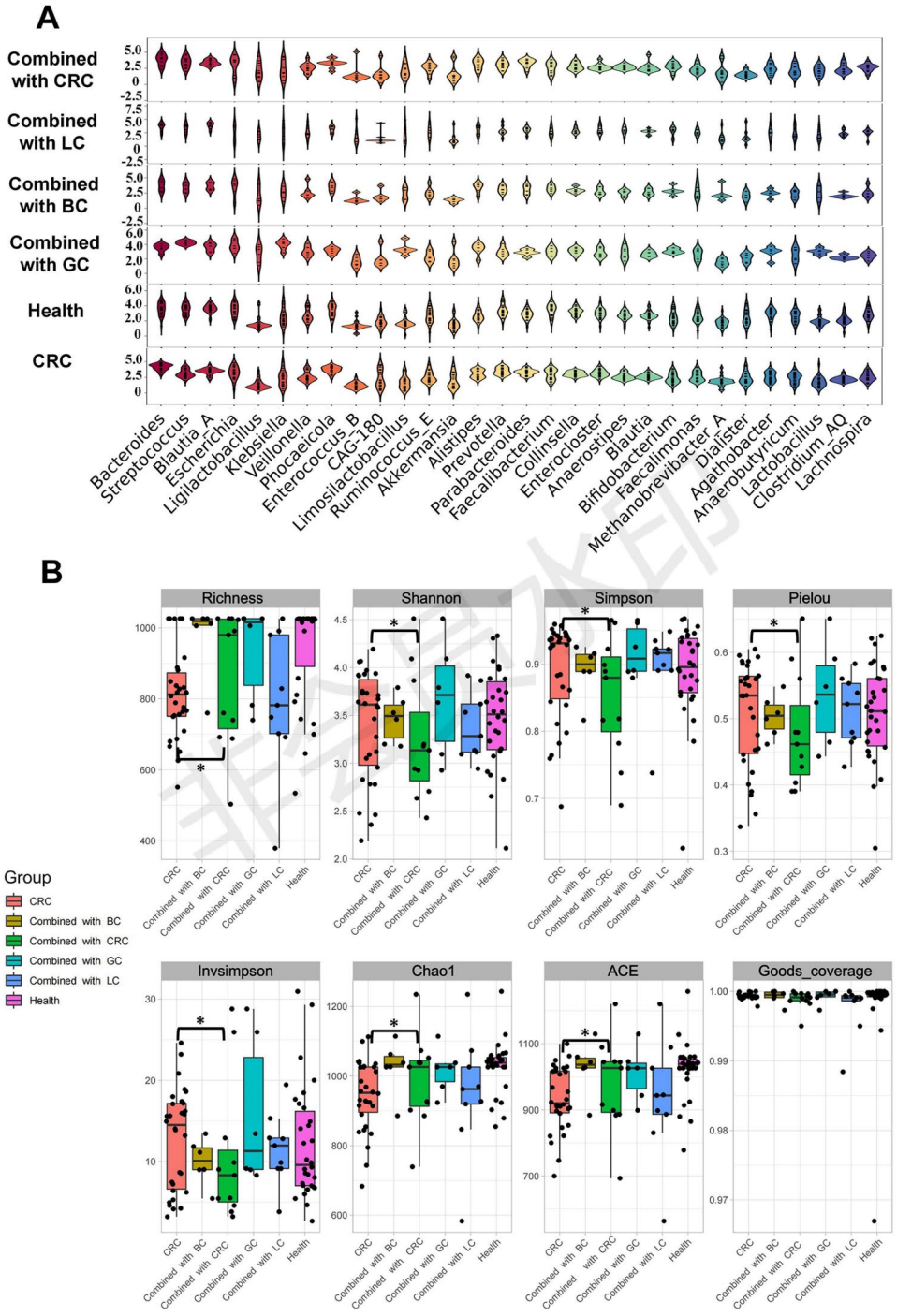

Fig. 2 Diversity analysis of the gut bacteria. **A**: The distributions of the top 30 gut bacteria with the highest abundance in different groups were shown in the violin figure. **B**: The boxplot of gut bacteria. Shanno, Simpson and Invsimpson represent gut bacteria diversity. Chao and ACE represent gut bacteria richness. Pielous stands for gut bacteria uniformity ($p < 0.05$)

bacteria were found between MPMs and healthy individuals. For instance, the abundance of *Dialister*, *Fecalibacterium*, *Mediterraneibacter* is significantly increased in healthy individuals compared with MPM (Fig. 4A). After that, we compared the abundance of gut bacteria between CRCs and MPM complicated by CRC patients. The results showed that 20 gut bacteria, including *Parvimonas*, *Dialister* and *Mediterraneibacter*, were more abundant in MPM complicated by CRC compared with CRCs (Fig. 4B). Then, the intersection of several significantly different bacteria groups was taken, and it was determined that the abundance of *Dialister* and

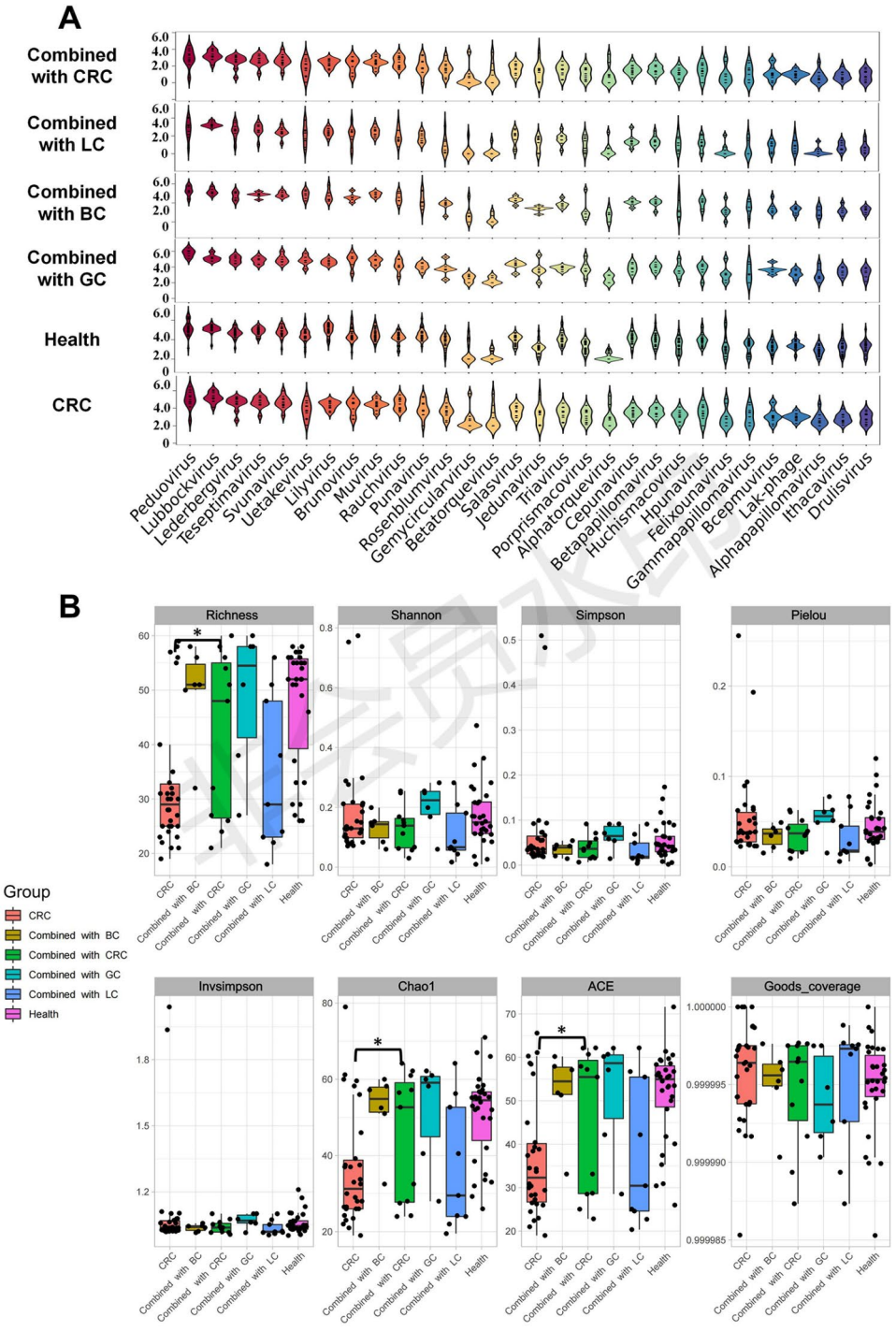

Fig. 3 Diversity analysis of the enterovirus. **A**: The distributions of the top 30 enterovirus with the highest abundance in different groups were shown in the violin figure. **B**: The boxplot of enterovirus. Shanno, Simpson and Invsimpson represent enterovirus diversity. Chao and ACE represent enterovirus richness. Pielous stands for enterovirus uniformity ($p < 0.05$)

Mediterraneibacter is lower in MPMs compared with healthy individuals, while that is lower in MPM complicated by CRC patients compared with CRCs. The abundance of *Dialister* and *Mediterraneibacter* is lower in MPM complicated by CRC compared with healthy individuals (Fig. 4C).

A total of 21 different enterovirus were screened between MPMs and healthy individuals. For example, the abundance of *Triavirus*, *Punavirus* and *Lilyvirus* is significantly increased in healthy individuals compared with MPM (Fig. 5A). In addition, the abundance of *Triavirus*, *Punavirus* and *Lilyvirus* in CRC patients were also

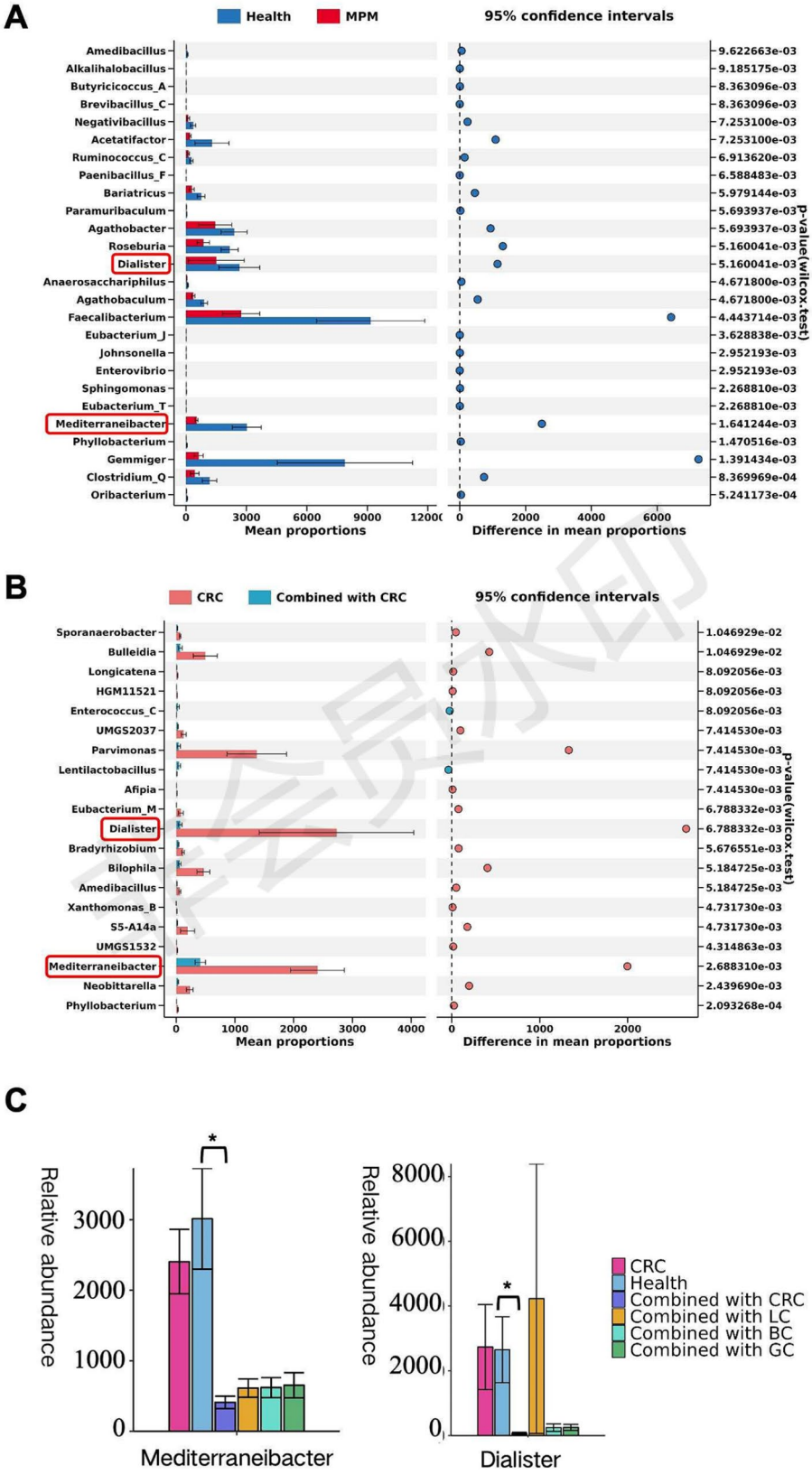

Fig. 4 Differential analysis of the gut bacteria. **A**: Differential analysis of gut bacteria in Health and MPM. **B**: Differential analysis of gut bacteria in CRC and complicated by CRC. **C**: Bar Figure of a comparison of the abundance of different gut bacteria (*Dialister* and *Mediterraneibacter*) in each group ($p < 0.05$)

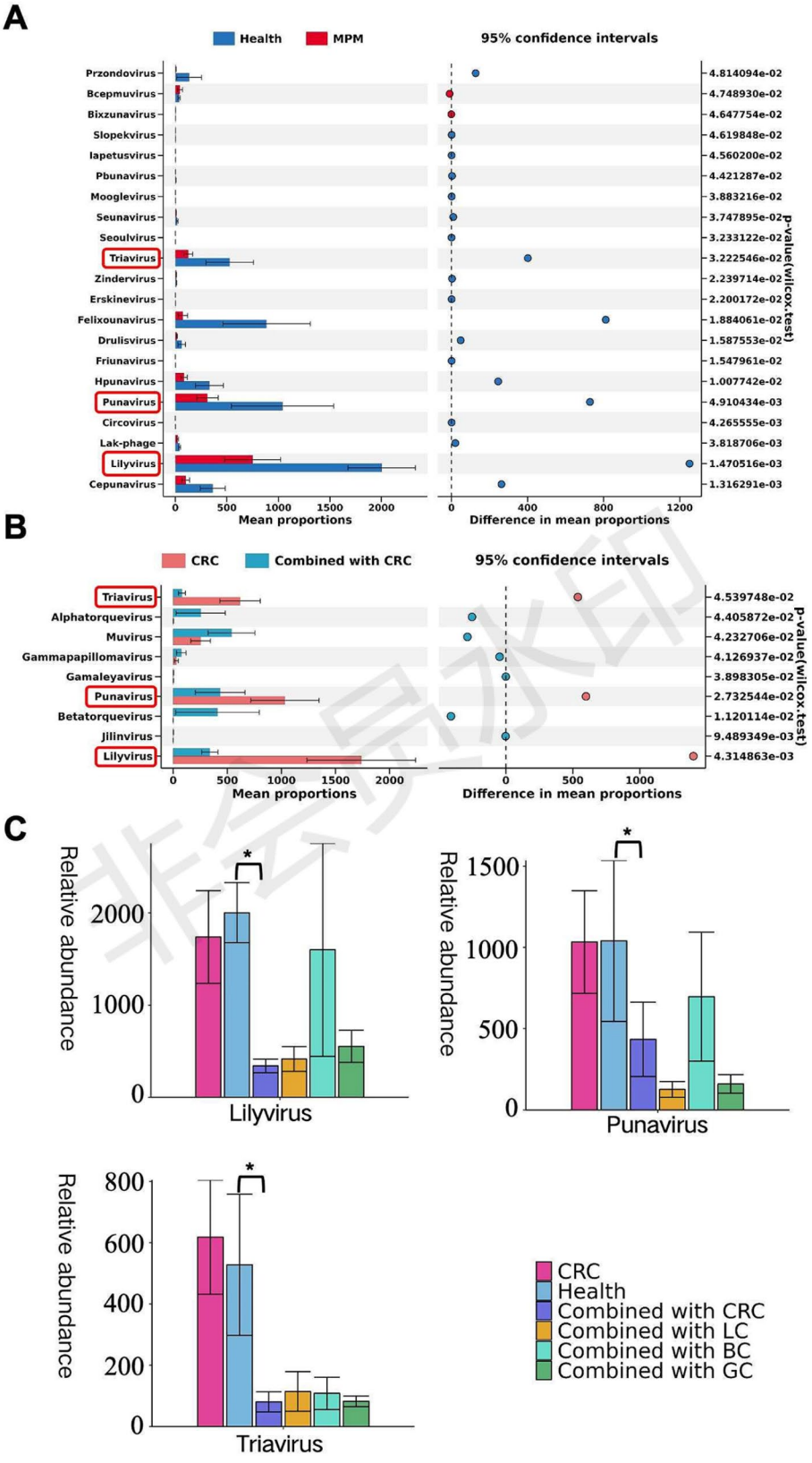

Fig. 5 Differential analysis of the enterovirus. **A**: Differential analysis of enterovirus in Health and MPM. **B**: Differential analysis of enterovirus in CRC and complicated by CRC. **C**: Bar Figure of a comparison of the abundance of different enterovirus (*Lilyvirus*, *Punavirus*, and *Triavirus*) in each group ($p < 0.05$)

lower than MPM complicated by CRC patients (Fig. 5B). Finally, the intersection of several viruses with significant differences was taken, and it was determined that the abundance of *Lilyvirus*, *Punavirus*, and *Triavirus* is lower in MPMs compared with healthy individuals, while that s lower in MPM complicated by CRC patients compared with CRCs. *Lilyvirus*, *Punavirus*, and *Triavirus* is more abundant in healthy individuals compared with MPM complicated by CRC (Fig. 5C).

Discussion

In this study, macrogene sequencing was used to conduct microbes component analysis, diversity analysis and difference analysis of gut microbiome in MPM patients, CRC patients and healthy individuals. The knowledge graph of gut microbiome (including gut bacteria and enterovirus) in MPM patients was plotted. We also found that the richness of gut microbiome is higher in MPM complicated by CRC patients compared with CRCs. The abundance of gut bacteria (*Dialister* and *Mediterraneibacter*), as well as enteroviruses (*Lilyvirus*, *Punavirus*, and *Triavirus*), is lower in MPMs compared to healthy individuals, and also lower in MPM complicated by CRC patients compared to CRCs. Considering that the gut species richness of MPM patients with CRC is higher than CRC, these 5 microbes are more scientific as microbial targets for MPM patients with CRC. Gut holds in the human body in the highest abundance and diversity of bacteria, including *Phylum Firmicutes*, *Bacteroides*, *Proteobacteria* and *Actinobacteria*, etc [29]. With the deepening of the study, the researchers gradually realized that the gut micro ecology system change is a tumor of inducing factors [30–33]. In summary, studying the relationship of MPM with gut microbes helps to provide basic data support for future studies of MPM.

The incidence of MPM patients is gradually increasing, and its relationship with gut microbes is still unclear. This study intends to innovatively plot the knowledge graph of gut microbes in MPM patients from the perspective of bacteria and virus through metagenomics technology, and explore the relationship between MPM and gut microbes. In addition, MPM patients complicated by CRC have a richer gut microbiome compared with CRC, but a total 5 microbial targets for MPM patients complicated by CRC, including *Dialister*, *Mediterraneibacter*, *Lilyvirus*, *Punavirus*, and *Triavirus*, were less abundant in MPM patients complicated by CRC. This result has been verified by our three analyses, and has high objectivity and scientificity. In fact, alterations in the gut microecosystem are important triggers and potentially beneficial contributors to cancer. The 5 microbes we identified that could potentially serve as potential targets may be the beneficial microbiome.

The genus *Dialister* is an anaerobe that produces propionate in the gut [36]. Doratha A Byrd et al. found that abundant *Dialister* was associated with improved overall survival in 452 CRC patients [34]. *Mediterraneibacte* is an anaerobic bacteria that produces short-chain fatty acids (SCFA) [35], including butyric acid, acetic acid, propionic acid and valoxalic acid, through fermentation of dietary fiber [36]. Both propionate and butyrate inhibit histone deacetylase [37] and butyrate has been confirmed to inhibit the proliferation of tumor cells and can be used to prevent and treat tumors [38–41]. This is similar to the conclusion of our conjecture that the protective effect of these two bacterial taxa on MPM may be related to SCFA, especially propionate and butyrate. However, the exact role remains unclear, and further investigation of the role of *Dialister* and *Mediterraneibacter* is warranted. In addition, there are few studies on the relationship between viruses and tumors now, and we hope that our research will provide a new direction in the deep study of the relationship between viruses and tumors.

In addition, this study reported changes in the abundance of *Triavirus*, *Punavirus* and *Lilyvirus* in MPM patients. Studies have found that viruses may promote the development of cancer by interfering with the cell cycle, inhibiting apoptosis, or activating carcinogenic pathways. Human papillomavirus (HPV) E6 and E7 proteins promote cell proliferation and inhibit apoptosis by interfering with p53, a key regulatory protein of host cells, thus inducing cervical cancer [42]. Epstein-barr virus (EBV) LMP1 activates PI3K/AKT/MTOR signaling pathway to induce up-regulation of B7-H3 expression, inhibit NK cell-mediated anti-tumor function, and thus promote the progression of nasopharyngeal carcinoma [43]. Hepatitis B virus (HBV) infection can lead to cirrhosis, which can progress to liver cancer. The role of viruses in tumorigenesis and treatment has gradually attracted attention in recent years. For example, oncolytic viruses (such as herpes simplex virus and adenovirus) have been used in cancer therapy, which exert anti-tumor effects by selectively infecting and lyzing tumor cells [44–45]. In addition, as an important component of the intestinal microbiome, enteroviruses may affect the tumor microenvironment by regulating host immune response or interacting with bacteria [46–47]. Although there is currently no direct evidence linking *Lilyvirus*, *Punavirus*, and *Triavirus* to tumors, our study provides new directions to explore the role of these viruses in tumorigenesis. Future studies could further explore whether these viruses influence tumor progression by regulating the gut microbiome or the host immune system.

The findings of this study not only provide a new perspective for the microbiome study of MPM, but also have important clinical application value and basic research potential. For example, *Dialister* and *Mediterraneibacter*

may be potential targets for cancer prevention and treatment through their metabolites, such as SCFAs. In addition, the in-depth study of enteroviruses may provide a new strategy for tumor immunotherapy. Future studies can be combined with multi-omics techniques (such as metabolomics and trace elements) to further reveal the specific mechanisms of these microorganisms and viruses in tumor occurrence and development, and provide a theoretical basis for personalized treatment.

However, this study has some limitations. First of all, the incidence rate of MPM is exceedingly low, approximately ranging from 2.4 to 17.2%, which makes the acquisition of a large sample size particularly difficult. We have included as many eligible patients as possible in the study to ensure that the sample is representative. Small sample sizes may limit statistical power and increase uncertainty in the results. Despite the limited sample size, our main findings were statistically significant ($p < 0.05$). We plan to expand the sample size in future studies, including collaboration with other research institutions, to obtain more patient data. We will explore multicenter study designs to further improve sample diversity and representation. Second, our findings were mainly based on differential analysis of microbiome sequencing data, and these results could only show a statistical association between changes in microbial abundance and tumor status, but could not directly infer causality, and lack experimental validation to identify microbial function. In the future, multiple omics data such as metabolomics and transcriptomics will be combined to fully reveal the interaction between microbes and MPM. In vitro cell experiments and animal models were used to verify the specific mechanism of action of these microorganisms in MPM. Finally, as an important environmental factor in tumor occurrence, gut microbiome is related to many factors such as diet and economic status. These factors were not included in this study due to difficulties in developing evaluation measures and collecting clinical data, as well as limitations in the duration of the study. We will systematically collect dietary data (such as food frequency questionnaires) and economic status information (such as income level, education, etc.) from participants in follow-up studies to more fully control for these confounding factors. In the future, more samples and clinical indicators will be included on the basis of the original, so as to provide more comprehensive information for the study of gut microbiome and MPM.

Conclusion

The knowledge graph of gut microbiome in MPM patients was plotted. There was a bigger number of gut microbes species(bacteria and virus) in MPM complicated by CRC patients compared with CRCs. A total of 5 characteristic gut microbes(*Dialister*, *Mediterraneibacter*, *Lilyvirus*, *Punavirus*, and *Triavirus*) had the potential to serve as biological markers of MPM complicated by CRC patients.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12885-025-13894-7.

Supplementary Material 1

Acknowledgements
Not applicable.

Author contributions
Quan Qi and Shuwen Han conceived and designed the study.Shuwen Han, Zhanbo Qu and Yinhang Wu wrote the manuscript.Zheng Wu and Jing Zhuang carried out the data acquisition, date analysis and statistical analysis. Yingchen Wang, Zefeng Wang and Jian Chu designed and draw figures.All authors read and approved the paper.

Funding
This work was supported by Zhejiang Provincial Clinical Research Center for CANCER (2022E50008, 2024ZY01056).

Data availability
The datasets generated for this study can be accessed from the China National GeneBank DataBase (CNGBdb), with the ID of CNP0004360 (https://db.cngb.org/search/project/CNP0004360/).

Declarations

Ethics approval and consent to participate
The patients' clinical protocol and informed consent were approved by the Ethics Committee of Huzhou Central Hospital (No. 202202005-02) and the Chinese Clinical Trial Registry (ChiCTR2100050167{Aug 19, 2021}||http://www.chictr.org.cn/).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 3 February 2025 / Accepted: 10 March 2025
Published online: 20 March 2025

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