Microbiome typing in uveal melanoma is associated with plaque radiotherapy

Yuning Chen

Beijing Tongren Hospital, Capital Medical University

Jingting Luo

Beijing Tongren Hospital, Capital Medical University

Haowen Li

Beijing Tongren Hospital, Capital Medical University

Rui Fang

Beijing Tongren Hospital, Capital Medical University

Ruiheng Zhang

Beijing Tongren Hospital, Capital Medical University

Yueming Liu

Beijing Tongren Hospital, Capital Medical University

Yang Li

Beijing Tongren Hospital, Capital Medical University

Wenbin Wei (✉ weiwenbin@mail.ccmu.edu.cn)

Beijing Tongren Hospital, Capital Medical University

Jingying Xiu

Beijing Tongren Hospital, Capital Medical University

Research Article

Keywords: Uveal melanoma, Microbiome, Plaque radiotherapy, Clinical characteristic

Posted Date: February 18th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1366478/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background: Microbiomes have been identified in various tumor types and could affect tumor progression and treatment. As the most prevalent primary malignant eye

tumor in adults, uveal melanoma (UM) has not been explored regarding its endogenous microbiome. Plaque radiotherapy (PRT) is the gold standard for the treatment of UM. Hereby, we recruited 71 UM patients, sequenced the 16S rRNA gene of their tumor tissues, and analyzed the association between UM microbiome and disease phenotypes.

Results: Clear bacterial signals were observed in UM tissues by using in situ fluorescence hybridization. 523 bacterial species passed strict decontamination against 58 environmental control samples in 16S rRNA gene analysis, and these species formed three distinct types by unsupervised clustering. The UM microbiome types were significantly associated with PRT. A biomarker analysis showed that *Pseudomonas* and *Sphingomonas* were significantly enriched in the radiation group (RG) compared to the non-radiation group (NRG). A kind of radiation-resistant bacteria had a significantly higher positive rate in tumor tissues that underwent radiotherapy. We found that radioresistant bacteria *Deinococcus* was associated with larger tumor and later tumor stage, while *Pedobacter*, *Bradyrhizobium*, *Variovorax*, *Pseudomonas* and *Stenotrophomonas* were associated with later metastasis.

Conclusion: There was an endogenous microbiome in UM tissues, and the distribution of microbiota was correlated with PRT and clinical features.

Introduction

Uveal melanoma (UM) is the most common primary intraocular malignancy in adults, with an annual incidence in Asian countries of 0.6/1000000[1, 2]. Due to the lack of a lymphatic system in the eye, UM cells enter the systemic circulation mainly through blood vessels in the eye, resulting in distant metastasis. Studies have shown that nearly 50% of patients develop metastases, with liver involvement more common, and nearly 80% of patients die within one year after metastasis[3]. At present, the treatment of UM is mainly divided into enucleation and eye-preserving therapy, in which plaque brachytherapy, a kind of plaque radiotherapy (PRT) is the main treatment[4].

The human microbiome is made up of microbes that live inside and on the surface of the human body. All humans share a core microbiome[5] in which the gut microbiome is influenced by differences in diet and host phenotype, thus diversifying the individual microbial ecosystem[6]. The gut microbiome has been shown to influence tumor biology in many ways, such as regulating inflammatory responses, inducing DNA damage, producing metabolites associated with tumorigenesis or tumor suppression, and responding to cancer treatment, including immunotherapy[7]. Previous studies have confirmed that the diversity of intestinal flora in melanoma patients receiving checkpoint blockade immunotherapy is significantly correlated with the therapeutic effect[8], while UM patients do not respond well to checkpoint blockade immunotherapy.

In addition to intestinal flora, bacteria have been found in breast cancer, lung cancer, cutaneous melanoma and other tumor tissues, and the composition of different types of tumor microbiome is different. The characteristics of tumor flora and metabolic pathway are related to tumor type and subtype, clinical characteristics of patients, tumor development and therapeutic effect[9, 10]. It is speculated that the bacteria selectively colonize tumors with abnormal vascular systems with abundant blood supply and relative leakage, thus growing and multiplying in the relatively hypoxic tumor microenvironment (especially anaerobe or facultative anaerobe). They can regulate enzyme activity, activate specific receptors on cancer cells to make them resistant to chemotherapy, and alter the tumor microenvironment through immune regulation[11]. A recent study found that oral vancomycin was associated with enhanced radiotherapy in cutaneous melanoma xenotransplantation models[12].

Based on the above research background, we speculate that there may be a microbiome in UM. Therefore, 71 cases of UM tissues were sequenced by 16S rRNA and analyzed in the present study. We want to explore the composition of the UM microbiota and the relationship between bacteria and PRT.

Results

Clinical Data of 71 UM samples

A total of seventy-one UM tissues were included in this study and 16S rRNA sequencing was performed, including 36 cases in radiation group (RG) and 35 cases in non-radiation group (NRG) (Fig. 1). Our cohort's average age of onset was younger than western patients, NRG was 50.7 ± 11.6 years and RG was 46.7 ± 11.5 years. 34 males and 37 females were included. There were 41 tumors in the right eye and 30 in the left eye. In this study, 7.2% of the tumors were in Stage I, 52.1% were in Stage II, and 40.6% were in Stage III (The American Joint Committee on Cancer classification, 8th). According to Collaborative Ocular Melanoma Study's recommendation[13], the tumor thickness was no more than 10.0mm could consider accepting PRT, so there was a significant difference in average tumor thickness between NRG ($10.7 \pm 3.8\text{mm}$) and RG ($6.2 \pm 2.6\text{mm}$) ($p < 0.001$), but there was no significant difference in the average largest basal diameter between the two groups (14.7 ± 4.4 vs $13.4 \pm 3.6\text{mm}$).

Tyramide signal amplification-fluorescence in situ hybridization detected bacteria in UM tissues

We performed tyramide signal amplification-fluorescence in situ hybridization (TSA-FISH) in 5 tissues (2 of NRG and 3 of RG) using the probe EUB338 I-III targeting bacterial 16S rRNA. At the same time, we also conducted a negative control experiment and found that the fluorescence signal of specific staining could not be observed without adding EUB probe. Staining for EUB338-probed hybridized sections were detected both in RG and NRG tissues, as shown in Fig. 2. Most bacteria are distributed in the cytoplasm and close to the nucleus. The positive bacterial signals were constantly observed in hybridized sections using the probe EUB338 I-III in all five patients.

Characterizations of UM microbiome in NRG and RG

A total of 71 tissue, including UM samples (35 of NRG and 36 of RG) and 58 environmental samples (operating room and laboratory environment) have been sent to undergo 16S rRNA gene sequencing (V3–V4 regions). We set four filtering criteria for algorithmic decontamination (threshold score of 0.01, 0.1, 0.5, 0.9, the lower the score, the stricter the decontamination degree) to remove potential contaminations from UM tissue using the forementioned environmental samples as contamination sources. After decontamination, we obtained the OTU number of the corresponding threshold score, respectively (Fig. 3A). To ensure the authenticity of the existence of bacteria, we used the OTU table with a decontamination threshold score of 0.01, removed the samples with reads less than 2000 (NR34, R21 and R26) and standardized analysis according to the minimum sequencing quantity of the remaining samples, which OTU number was 523 and had 160956 reads as the basis for further analysis.

We hope to observe the OTU number and relative abundance of all kinds of bacteria in NRG and RG at different species classification levels. The results showed that the UM tissue was dominated by *Proteobacteria* at the phylum level, while in some RG samples, the abundance of *Firmicutes* and *Bacteroidetes* was relatively high (Fig. 3B). At the family and genus level, the abundance of different species in each sample was relatively stable (Figs. 3C and 3D). The relative abundance of *Stenotrophomonas* in 11 RG samples was more than 50%, while only 6 in NRG. We found that there are only 17 species whose relative abundance was greater than 0.01 and can be classified at the genus level. They were *Stenotrophomonas*, *Variovorax*, *Pedobacter*, *Pseudomonas*, *Acinetobacter*, *Micrococcus*, *Elizabethkingia*, *Aerococcus*, *Deinococcus*, *Leptothrix*, *Comamonas*, *Enhydrobacter*, *Sphingomonas*, *Bradyrhizobium*, *Methylobacterium*, *Brevundimonas* and *Flavisolibacter*. The results showed that the main component of UM microbiome is *Proteobacteria*, including *Stenotrophomonas* and *Variovorax*.

Microbiota types in UM tissues

We referred to the enterotype classification method of the gut microbiota[14] and performed it in UM samples. The UM samples formed three clusters that we designate them as UM ‘Types’ (Fig. 4, **Supplement Fig. 1**). Type2 markers were *Stenotrophomonas*, *Methylobacterium*, *Pseudomonas*, *Elizabethkingia*, *Pedobacter*, *Variovorax*, *Sphingomonas* and *Comamonas* (**Supplement Fig. 1A-H**), the enrichment of these bacteria in Type2 was significantly higher than that of Type1 and Type3. Although we did not find any bacterial taxonomy that is specifically enriched in Type1 or Type3, we compared the relative abundance of 17 species and ‘others’ in RG and NRG, and found that the relative abundance of *Pseudomonas* and *Sphingomonas*, which were enriched in Type2, was also higher in RG (**Supplement Fig. 2A and 2B**), and the number of samples in RG in Type2 is more than that in NRG (Fig. 4A).

Differential expression and correlation network of bacteria in NRG and RG

Under the strictest threshold (0.01) of decontamination, comparison of the alpha and beta diversity indices showed no significance between NRG and RG. Additionally, linear discriminant analysis effect size (LEfSe) analysis showed that *Pseudomonas* and *Sphingomonas* were significantly higher in RG than that in NRG (linear discriminant analysis (LDA) score > 2.0, $p < 0.05$) (Fig. 4C). We further investigated the correlation of bacteria in RG and NRG at the genus level. The inclusion criteria with correlation coefficient over 0.4 were selected and presented in the form of the network diagram in Fig. 4D. Although there was only positive correlation among all species except ‘others’ in the two groups, it was still found that the bacteria were divided into two clusters in the RG. In the correlation network, *Stenotrophomonas*, with the highest abundance, has a positive correlation with only one cluster (left).

A. Composition of RG and NRG samples in each type. B. Types of UM samples which were visualized by PCoA and clustering at the genus level. C. Significant different bacteria enrichment between NRG and RG. The results could be biomarkers in RG. LDA score > 2.0, $p < 0.05$. D. Co-occurrence networks of NRG and RG at the genus level. The size of the circle represents the abundance of bacteria. The thickness of the connected lines represents the strength of the relationship between bacteria.

Correlating the UM microbiota composition with clinical characteristics

In order to investigate possible correlations between the microbiota composition of UM tissues and clinical characteristics, we included clinical features that may be related to the severity of the disease, calculated their associations with different bacteria, and inferred whether a species is related to the progression of the disease (Fig. 5, Table 1). We observed that *Micrococcus*, *Methylobacterium*, *Sphingomonas*, *Variovorax*, and *Pseudomonas* were correlated with younger patients (adjust $p < 0.05$). For tumor characteristics, tumor thickness was found to be negatively correlated with *Sphingomonas*, *Variovorax*, *Pseudomonas* and *Deinococcus* (adjust $p < 0.05$).

In analyses related to clinical outcomes, *Pedobacter*, *Bradyrhizobium*, *Variovorax*, *Pseudomonas* and *Stenotrophomonas* were associated with later metastasis, while *Variovorax* was related to a shorter metastasis-death time (adjust $p < 0.05$). More basic experiments and clinical trials will be needed to verify our results.

Table 1
Clinical prognosis characteristic of NRG(n = 35) and RG(n = 36).

Clinical prognosis characteristic	NRG	RG	P-value
Operation-Death Time (months)	13.8 ± 4.8	43.8 ± 13.4	<0.001
Operation-Metastasis Time (months)	13.0 ± 19.1	45.7 ± 19.5	0.004
Metastasis-Death Time (months)	9.8 ± 3.0	7.9 ± 6.7	0.613

Discussion

With the improvement of microbial detection technology, scientists have paid more and more attention to the research of flora. In recent years, variates studies have found that the existence of bacteria in tumor and it is related to the occurrence and development of disease[9, 15]. In this study, we found the existence of bacteria in UM for the first time, explained the characteristics of UM microbiota and its relationship with PRT and clinical features. It remains controversial whether bacteria are present in tissues previously thought sterile. The first conceptual evidence for bacterial translocation was reported in 2011 when Amar et al showed that bacterial 16S rDNA gene was detectable in blood[16]. After tumorigenesis, the destruction of physical and molecular barriers, coupled with relative immunosuppression in tumor microenvironment, may increase the likelihood of bacterial translocation to normally aseptic sites. Then various studies supported the hypothesis of translocation bacteria[17, 18]. A recent study found that bacteria are widespread in different types of tumors (including exposed and protected), of which melanoma has the least bacterial content[9]. We first presented the composition of the human UM microbiota and found that it could be divided into three types in the present study. We also observed differences in UM microbiota between NRG and RG, and evidenced that bacterial signatures were significantly associated with UM clinical characteristics.

Even though we use strict aseptic procedures during the operation, we still collect a large number of environmental control samples for algorithmic decontamination. After using the most stringent decontamination threshold of 0.01, we removed about 95% of the species, but there were still real bacterial signals, so the conclusion that bacteria were found in UM tissues in this research is credible. This study detected positive bacterial signals in both NRG and RG UM samples using TSA-FISH, distributing in cell plasm but not in nuclear.

After 16s rRNA sequencing and OTU annotation, we found that the UM microbiota was mainly *Proteobacteria*, and at the genus level, it was mainly *Stenotrophomonas*, *Variovorax* and *Pedobacter*. *Stenotrophomonas* has the highest relative abundance in UM microbiome, which is consistent with recent report[15]. Previous studies have shown that as a new opportunistic pathogen, patients with long-term neutropenia and exposure to broad-spectrum antibiotics have a higher risk of *Stenotrophomonas* infected, leading to pneumonia, bacteremia, catheter-related infection and other diseases[19]. There were more annotated species in RG with relative abundance > 0.01 than in NRG. While the proportion of 'other' in some samples was very high, which may be due to the following reasons: First, the species diversity was high, but the relative abundance was less than 0.01. Second, some species cannot be classified to the level of family or genus. Third, there may be new species that have not been discovered before.

A very interesting phenomenon is that all the UM samples formed three distinct clusters, which was in accordance with studies in gut enterotypes[14]. Among them, RG accounted for more in Type 2. To explore the microbiome characteristics of Type 2, we compared the relative abundance of all detected bacteria in the three types, and found that *Stenotrophomonas*, *Pseudomonas* and *Variovorax* were enriched in Type 2 and had statistical significance (**Supplement Fig. 1**, $p < 0.0001$). A study had shown that *Stenotrophomonas* can secrete outer membrane vesicles and elicit a potent inflammatory respons[20]. Lipopolysaccharide from *Pseudomonas solanacearum* possessed the expressed

antimetastatic effect in melanoma B-16 experimental animal[21]. The abundance of *Variovorax paradoxus* was correlated with histological gastritis[22]. Although *Bradyrhizobium*, *Enhydrobacter*, *Leptothrix* and *Acinetobacter* are abundant in Type3, they are different among the three types.

To explore the effect of PRT on UM microbiota, we analyzed RG and NRG by LEfSe to find out the difference between the two groups. The results showed that the abundances of *Pseudomonas* and *Sphingomonas* were higher in RG. Previous research showed that *Pseudomonas aeruginosa* ExoT induces G1 cell cycle arrest in melanoma cells, which significantly inhibits the growth of melanoma[23]. *Sphingomonas* was found to be enriched in tumor tissues in both thyroid cancer[24] and bladder cancer[25], while *Sphingomonas* was relatively enriched in normal breast tissues compared with breast cancer tissues[26]. We found that there was a significant difference in the positive rate of *Deinococcus* with strong radiation resistance[27] between RG (17 of 36 cases) and NRG (8 of 35 cases) ($p = 0.032$). Therefore, we thought that PRT influences UM microbiome.

We analyzed the correlation between 17 species and clinical information to explore the influence of UM microbiota on clinical characteristics and disease prognosis. *Deinococcus radiodurans* can survive in various environments with extreme radioactive and ultraviolet radiation[28], its extract Deinoxanthin can induce apoptosis of cancer cells[29]. This study found that *Deinococcus* was associated with small and early tumors, while the positive rate of *Deinococcus* in RG was high, and the operation-metastasis time and operation-death time in patients with RG was significantly longer than that in NRG (Table 1), suggesting that *Deinococcus* may play a role in inhibiting tumor development. *Pedobacter*[30] and *Bradyrhizobium*[10] have been confirmed to have anti-tumor effects in previous studies, which is consistent with our findings that they are positively correlated with late metastasis of UM. Although *Variovorax* was associated with better clinical phenotype before tumor metastasis, it was negatively correlated with metastasis-death time (the higher relative abundance of *Variovorax*, the faster the metastatic patients die). *Stenotrophomonas* was found to be associated with a better prognosis in this study, but it was thought in previous studies that it could elicit a strong inflammatory response[20] and enrich in tumor tissue[31], which is inconsistent with our conclusion and needs further experimental verification.

There were still some limitations in this study. First, the tumor tissue is small and lacks adjacent tissue samples. Due to the small size of the eyeball, the limitation of local excision, and the improvement of the eye preservation rate of UM, we can rarely obtain tumor and adjacent samples, especially the normal control samples. Second, lack of detection of living bacteria and metabolic function. Although we have detected bacteria in 16s rRNA and TSA-FISH, we cannot directly observe bacteria. We can add electron microscope detection in the future study, supplemented with living bacterial biomarkers and bacterial culture to verify the existence of living bacteria, which is a great challenge, because it requires very strict experimental conditions and techniques. Third, we cannot determine the source of the bacterial DNA detected in UM. Whether the bacteria were derived from the gut or adjacent sites, we need more types of samples and experiments for further exploration. Last, the analysis of UM tissues was based on the strictest threshold of decontamination, which actually may lose real bacterial signal. However, how to set

the decontamination threshold to maintain the authenticity of the sequencing results best and eliminate the interference of environmental contamination is still controversial. Nevertheless, previous study has shown that the number of bacteria in cutaneous melanoma is also the lowest compared to other types of tumors[9]. Therefore, more large-scale basic research and bioinformatics analysis are needed to explore whether the UM microbiome plays a causal role in UM occurrence, treatment and progression.

Conclusions

In conclusion, our study provides evidence for the existence and composition of bacteria in UM, exploring the relationship between UM's microbiome and the clinical characteristics. There are differences in tumor microbiota in UM patients treated with PRT or not, and UM could be divided into three types according to the microbiome, which is associated with PRT.

Methods

We chose the tumor tissues of 71 patients treated in Beijing Tongren Hospital. All samples were obtained by local resection of the tumor or enucleation. We divided 71 tumor samples into two groups according to whether they received PRT (RG) or not (NRG), and 16s rRNA sequencing and analysis were carried out. In addition, we selected UM tissues from 5 patients for tyramide signal amplification-fluorescence in situ hybridization (TSA-FISH), including 3 cases of RG and 2 cases of NRG. All samples were collected under aseptic conditions during the operation. At the same time, 58 environmental negative control samples were collected, including operating room air, sterile clothes, dry gauzes, wet gauzes, instrument washing solution, sterilized skin swabs, physiological saline, sterile towel swabs, sampling tubes, aseptic buffer solutions, laboratory air, sterile water and other environmental samples. The specimens were transferred directly from the operating room to the biosafety cabinet in the aseptic container in the 20 minutes. All samples were stored at -80°C until undergoing TSA-FISH or 16S rRNA sequencing. The main diagnostic and treatment criteria are consistent with the Collaborative Ocular Melanoma Study^[32]. We recorded demographic information (age, sex), eye and tumor features (best-corrected visual acuity, intraocular pressure, laterality, tumor size, shape, pigmentation, location, optic disc involvement, association with subretinal fluid, intraocular hemorrhage, ciliary body involvement and pathological types). Whether or not to receive PRT depends on the characteristics of the tumor^[4] and the patient's will. Otherwise, we recorded the time of tumor metastasis and death.

TSA-FISH

TSA-FISH was performed following a modified protocol described by Choi et al[33]. The basic steps included paraffin section dewaxing and tissue rehydrating, pretreatments of tissue sections, hybridizing probe and detection with anti-digoxigenin/horseradish peroxidase antibodies (anti-DIG HRP-conjugated antibody). EUB338 I-III were specific bacterial probes that could bind to the 16S rRNA region of most bacteria. Digoxin labeled EUB probe was selected to label bacteria in UM tissues. Anti-digoxin/horseradish peroxidase antibody was used as a secondary antibody to bind to EUB probe and

was observed under the same fluorescence microscope after amplification of Tyramine signal. All steps were performed under sterile conditions.

16S rRNA gene amplification and sequencing

Microbial DNA was extracted from UM samples (36 of RG and 35 of NRG) and negative samples (58 of environmental samples) using a DNA extraction kit according to the manufacturer's protocols. The concentration and purity were measured using the NanoDrop One (Thermo Fisher Scientific, MA, USA). Next, 16S rRNA genes of V3-V4 hypervariable regions were amplified with the primers 338F (5'-ACTCCTACGGGAGGCAGCA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') using the thermocycler PCR instrument BioRad S1000 (Bio-Rad Laboratory, CA, USA). The length and concentration of the PCR product were detected by 1% agarose gel electrophoresis. Samples with a bright main strip between could be used for further experiments. PCR products were mixed in equidensity ratios according to the GeneTools Analysis Software (V4.03.05.0, SynGene). Then, the mixture of PCR products was purified with E.Z.N.A. Gel Extraction Kit (Omega, USA). After purification of PCR products, Sequencing libraries were generated using NEBNext® Ultra™ DNA Library Prep Kit for Illumina® (New England Biolabs, MA, USA). The library quality was assessed on the Qubit® 2.0 Fluorometer (Thermo Fisher Scientific, MA, USA) and sequenced on Illumina Novaseq6000 System with Mode PE250 and 250 bp paired-end reads were generated.

Decontamination and data analysis

All 16S rRNA gene datasets were processed using a standardized pipeline in QIIME 2[34]. For each dataset, demultiplexed sequencing reads from the UM tissues and environmental control samples were denoised to generate amplicon sequence variants (ASVs) using the software DADA2 (V1.6)[35].

The Bayesian approach based SourceTracker(V0.9.1)[36] was used to estimate source proportions in UM samples that came from environment according to the species community structure of Sink(tumor samples) and Source(environmental samples). Another decontaminative software Decontam(V1.10.0) (<https://github.com/benjjneb/decontam>), an open-source R package that implements a statistical classification procedure that identifies contaminants in 16S data, was used to filter out features on genus level with a high chance of being contaminants using sequenced environmental control samples[37]. Prevalence based contaminant identification was used with four different threshold scores of 0.01, 0.1, 0.5, 0.9, respectively. We removed mitochondria, chloroplasts, unclassified species and the samples with reads less than 2000, then standardized analysis according to the minimum sequencing quantity of the remaining samples. After combining the above-mentioned methods, we obtained four OTU tables with different decontaminative levels.

Clustering and classification were performed using Jensen–Shannon distance and partitioning around medoid (PAM) clustering. The optimal number of clusters was estimated using the Calinski–Harabasz index[14].

Alpha diversity was applied in analyzing the complexity of species diversity for a sample through four indices, including Observed species, Chao1, Shannon, Ace, based on final OTU tables. All these indices in our samples were calculated with QIIME 2 and displayed with R software(V2.15.3).

Beta diversity analysis was used to evaluate differences of samples in species complexity. Principal Coordinate Analysis (PCoA) was performed to get principal coordinates and visualize from complex, multidimensional data. PCoA analysis was displayed by QIIME 2 and ggplot2 package in R software. NMDS analysis was performed by the vegan package of R software based on the weighted and unweighted unifracs distance matrix.

LEfSe analysis was used to find the biomarker of NRG and RG based on the final OTU table with threshold score of 0.01. Non-parametric factorial Kruskal-Wallis (KW) sum-rank test detected species with significant differences in abundance between different groups, and Wilcoxon rank sum test was used to judge the difference between the two groups. Finally, LDA was used to achieve dimensionality reduction and evaluate the impact of significantly different species (LDA Score) by setting LDA score ≥ 2 and obtained the biomarkers in different groups.

Correlation analysis and variation partitioning analysis were performed by R software based on the key genus (relative abundance $\geq 1\%$), and canonical correspondence analysis was analyzed based on the final OTU table with threshold score of 0.01. Spearman correlation index between species or samples based on the OTU table was calculated by R software and visualized by cytoscape (<http://cytoscape.org/>).

Correlations of the taxonomy counts (phylum and genus relative abundances) and disease features, such as demographic information, clinical features, and follow-up results were estimated using the Spearman correlation coefficient and represented in a correlation plot, using the R Corrplot package.

Statistical analysis

The statistical analyses were performed using the Statistical Product and Service Solutions (SPSS) software version 25 (International Business Machines Corporation, Armonk, New York, United States). Students' t-test was used to determine the difference between two groups. The positive rate of *Deinococcus* in RG and NRG was analyzed by chi-square test. Statistical significance was considered when p-value was less than 0.05.

Abbreviations

UM
Uveal melanoma
PRT
plaque radiotherapy
RG
Radiation group

NRG
Non-radiation group
LDA
Linear discriminant analysis
LEfSe
Linear discriminant analysis effect size
TSA-FISH
tyramide signal amplification-fluorescence in situ hybridization

Declarations

Ethics approval and consent to participate

This research adhered to the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board of Beijing Tongren Hospital. Furthermore, the study obtained the informed consent of all patients.

Consent for publication

Not applicable.

Availability of data and materials

Raw data of all samples were successfully uploaded on NCBI and the data will be released at <https://www.ncbi.nlm.nih.gov/sra/PRJNA807130> to the public on June. 30th, 2023.

Competing of interests

The authors declare that they have no competing interests.

Funding

This study was supported by The Capital Health Research and Development of Special (2020-1-2052), Science & Technology Project of Beijing Municipal Science & Technology Commission (Z201100005520045, Z181100001818003), The National Natural Science Foundation of China (82101180), Beijing Natural Science Foundation (7204245), Scientific Research Common Program of Beijing Municipal Commission of Education (KM202010025018), Beijing Municipal Administration of Hospitals' Youth Programme (QMS20190203), Beijing Dongcheng District Outstanding Talents Cultivating Plan(2018).

Authors' contributions

YNC, YL and WBW initiated and led the study. JTL, RF, RHZ and YML collected tumor tissues. YNC, JTL, HWL analyzed the data and wrote the manuscript. JYX uploaded 16S rRNA sequencing data to NCBI

database. All authors read and approved the final manuscript.

Acknowledgments

The authors thank those who participated in the present study.

Authors' information

¹Beijing Tongren Eye Center, Beijing Key Laboratory of Intraocular Tumor Diagnosis and Treatment, Beijing Ophthalmology & Visual Sciences Key Lab, Medical Artificial Intelligence Research and Verification Key Laboratory of the Ministry of Industry and Information Technology, Beijing Tongren Hospital, Capital Medical University, Beijing, 100730, P. R. China

References

1. Tomizuka T, Namikawa K, Higashi T. Characteristics of melanoma in Japan: a nationwide registry analysis 2011-2013. *Melanoma Res.* 2017;27(5):492-7. <http://dx.doi.org/10.1097/CMR.0000000000000375>
2. Park SJ, Oh CM, Kim BW, Woo SJ, Cho H, Park KH. Nationwide Incidence of Ocular Melanoma in South Korea by Using the National Cancer Registry Database (1999-2011). *Invest Ophthalmol Vis Sci.* 2015;56(8):4719-24. <http://dx.doi.org/10.1167/iovs.15-16532>
3. Jager MJ, Shields CL, Cebulla CM, Abdel-Rahman MH, Grossniklaus HE, Stern MH, et al. Uveal melanoma. *Nat Rev Dis Primers.* 2020;6(1):24. <http://dx.doi.org/10.1038/s41572-020-0158-0>
4. Chen YN, Wang YN, Chen MX, Zhang K, Chen RT, Fang R, et al. Machine learning models for outcome prediction of Chinese uveal melanoma patients: A 15-year follow-up study. *Cancer Commun (Lond).* 2022. <http://dx.doi.org/10.1002/cac2.12253>
5. Turnbaugh PJ, Hamady M, Yatsunencko T, Cantarel BL, Duncan A, Ley RE, et al. A core gut microbiome in obese and lean twins. *Nature.* 2009;457(7228):480-4. <http://dx.doi.org/10.1038/nature07540>
6. Structure, function and diversity of the healthy human microbiome. *Nature.* 2012;486(7402):207-14. <http://dx.doi.org/10.1038/nature11234>
7. Bhatt AP, Redinbo MR, Bultman SJ. The role of the microbiome in cancer development and therapy. *CA Cancer J Clin.* 2017;67(4):326-44. <http://dx.doi.org/10.3322/caac.21398>
8. Gopalakrishnan V, Spencer CN, Nezi L, Reuben A, Andrews MC, Karpinets TV, et al. Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. *Science.* 2018;359(6371):97-103. <http://dx.doi.org/10.1126/science.aan4236>
9. Nejman D, Livyatan I, Fuks G, Gavert N, Zwang Y, Geller LT, et al. The human tumor microbiome is composed of tumor type-specific intracellular bacteria. *Science.* 2020;368(6494):973-80. <http://dx.doi.org/10.1126/science.aay9189>
10. Ma J, Gnanasekar A, Lee A, Li WT, Haas M, Wang-Rodriguez J, et al. Influence of Intratumor Microbiome on Clinical Outcome and Immune Processes in Prostate Cancer. *Cancers (Basel).*

- 2020,12(9). <http://dx.doi.org/10.3390/cancers12092524>
11. Helmink BA, Khan MAW, Hermann A, Gopalakrishnan V, Wargo JA. The microbiome, cancer, and cancer therapy. *Nat Med*. 2019,25(3):377-88. <http://dx.doi.org/10.1038/s41591-019-0377-7>
 12. Liu J, Liu C, Yue J. Radiotherapy and the gut microbiome: facts and fiction. *Radiat Oncol*. 2021,16(1):9. <http://dx.doi.org/10.1186/s13014-020-01735-9>
 13. Jampol LM, Moy CS, Murray TG, Reynolds SM, Albert DM, Schachat AP, et al. The COMS Randomized Trial of Iodine 125 Brachytherapy for Choroidal Melanoma: IV. Local Treatment Failure and Enucleation in the First 5 Years after Brachytherapy. COMS Report No. 19. *Ophthalmology*. 2020,127(4S):S148-S57. <http://dx.doi.org/10.1016/j.ophtha.2020.01.032>
 14. Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR, et al. Enterotypes of the human gut microbiome. *Nature*. 2011,473(7346):174-80. <http://dx.doi.org/10.1038/nature09944>
 15. Liu B, Zhou Z, Jin Y, Lu J, Feng D, Peng R, et al. Hepatic stellate cell activation and senescence induced by intrahepatic microbiota disturbances drive progression of liver cirrhosis toward hepatocellular carcinoma. *J Immunother Cancer*. 2022,10(1). <http://dx.doi.org/10.1136/jitc-2021-003069>
 16. Amar J, Serino M, Lange C, Chabo C, Iacovoni J, Mondot S, et al. Involvement of tissue bacteria in the onset of diabetes in humans: evidence for a concept. *Diabetologia*. 2011,54(12):3055-61. <http://dx.doi.org/10.1007/s00125-011-2329-8>
 17. Cummins J, Tangney M. Bacteria and tumours: causative agents or opportunistic inhabitants? *Infect Agent Cancer*. 2013,8(1):11. <http://dx.doi.org/10.1186/1750-9378-8-11>
 18. Baban CK, Cronin M, O'Hanlon D, O'Sullivan GC, Tangney M. Bacteria as vectors for gene therapy of cancer. *Bioeng Bugs*. 2010,1(6):385-94. <http://dx.doi.org/10.4161/bbug.1.6.13146>
 19. Safdar A, Rolston KV. *Stenotrophomonas maltophilia*: changing spectrum of a serious bacterial pathogen in patients with cancer. *Clin Infect Dis*. 2007,45(12):1602-9. <http://dx.doi.org/10.1086/522998>
 20. Kim YJ, Jeon H, Na SH, Kwon HI, Selasi GN, Nicholas A, et al. *Stenotrophomonas maltophilia* outer membrane vesicles elicit a potent inflammatory response in vitro and in vivo. *Pathog Dis*. 2016,74(8). <http://dx.doi.org/10.1093/femspd/ftw104>
 21. Varbanets LD, Shmal'ko Iu P, Pridatko OE, Zhukova EV, Moskalenko NV. [The biological activity of *Pseudomonas solanacearum* polysaccharide]. *Mikrobiol Z*. 1995,57(2):80-5.
 22. Han HS, Lee SY, Oh SY, Moon HW, Cho H, Kim JH. Correlations of the Gastric and Duodenal Microbiota with Histological, Endoscopic, and Symptomatic Gastritis. *J Clin Med*. 2019,8(3). <http://dx.doi.org/10.3390/jcm8030312>
 23. Mohamed MF, Wood SJ, Roy R, Reiser J, Kuzel TM, Shafikhani SH. *Pseudomonas aeruginosa* ExoT induces G1 cell cycle arrest in melanoma cells. *Cell Microbiol*. 2021,23(8):e13339. <http://dx.doi.org/10.1111/cmi.13339>
 24. Dai D, Yang Y, Yang Y, Dang T, Xiao J, Wang W, et al. Alterations of thyroid microbiota across different thyroid microhabitats in patients with thyroid carcinoma. *J Transl Med*. 2021,19(1):488.

<http://dx.doi.org/10.1186/s12967-021-03167-9>

25. Liu F, Liu A, Lu X, Zhang Z, Xue Y, Xu J, et al. Dysbiosis signatures of the microbial profile in tissue from bladder cancer. *Cancer Med*. 2019;8(16):6904-14. <http://dx.doi.org/10.1002/cam4.2419>
26. Xuan C, Shamonki JM, Chung A, Dinome ML, Chung M, Sieling PA, et al. Microbial dysbiosis is associated with human breast cancer. *PLoS One*. 2014;9(1):e83744. <http://dx.doi.org/10.1371/journal.pone.0083744>
27. Lim S, Jung JH, Blanchard L, de Groot A. Conservation and diversity of radiation and oxidative stress resistance mechanisms in *Deinococcus* species. *FEMS Microbiol Rev*. 2019;43(1):19-52. <http://dx.doi.org/10.1093/femsre/fuy037>
28. Rew DA. *Deinococcus radiodurans*. *Eur J Surg Oncol*. 2003;29(6):557-8. [http://dx.doi.org/10.1016/s0748-7983\(03\)00080-5](http://dx.doi.org/10.1016/s0748-7983(03)00080-5)
29. Choi YJ, Hur JM, Lim S, Jo M, Kim DH, Choi JI. Induction of apoptosis by deinoxanthin in human cancer cells. *Anticancer Res*. 2014;34(4):1829-35.
30. Rani A, Rajulapati V, Goyal A. Antitumor effect of chondroitin AC lyase (PsPL8A) from *Pedobacter saltans* on melanoma and fibrosarcoma cell lines by in vitro analysis. *Pharmacol Rep*. 2019;71(1):167-74. <http://dx.doi.org/10.1016/j.pharep.2018.10.009>
31. Shojaee S, Sharma A, Gottel N, Sanchez T, Gilbert JA, Rahman NM. Microbiome profile associated with malignant pleural effusion. *PLoS One*. 2020;15(5):e0232181. <http://dx.doi.org/10.1371/journal.pone.0232181>
32. Accuracy of diagnosis of choroidal melanomas in the Collaborative Ocular Melanoma Study. COMS report no. 1. *Arch Ophthalmol*. 1990;108(9):1268-73. <http://dx.doi.org/10.1001/archopht.1990.01070110084030>
33. Choi YS, Kim YC, Baek KJ, Choi Y. In Situ Detection of Bacteria within Paraffin-embedded Tissues Using a Digoxin-labeled DNA Probe Targeting 16S rRNA. *J Vis Exp*. 2015(99):e52836. <http://dx.doi.org/10.3791/52836>
34. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat Biotechnol*. 2019;37(8):852-7. <http://dx.doi.org/10.1038/s41587-019-0209-9>
35. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods*. 2016;13(7):581-3. <http://dx.doi.org/10.1038/nmeth.3869>
36. Knights D, Kuczynski J, Charlson ES, Zaneveld J, Mozer MC, Collman RG, et al. Bayesian community-wide culture-independent microbial source tracking. *Nat Methods*. 2011;8(9):761-3. <http://dx.doi.org/10.1038/nmeth.1650>
37. Davis NM, Proctor DM, Holmes SP, Relman DA, Callahan BJ. Simple statistical identification and removal of contaminant sequences in marker-gene and metagenomics data. *Microbiome*. 2018;6(1):226. <http://dx.doi.org/10.1186/s40168-018-0605-2>

Figures

Figure 1

Overview of the technical route in the current study.

Figure 2

Visualization of bacteria using TSA-FISH. Representative in situ detection of bacteria obtained by TSA-FISH using DAPI staining (in blue) and HRP-labelled EUB338 I-III probe mixture (in green), showing the presence and distribution of specific hybridized bacterial signals(arrow) in UM tissue. Original magnification: $\times 100.0$, scale bar=10 μ m. A. UM tissue of NRG (n=2), b. UM tissue of RG (n=3).

Figure 3

Effect of algorithmic decontamination and composition of UM microbiome. A. OTU number histogram with different degrees of decontamination. Histogram of All samples (top), NRG (median) and RG (bottom). B-D. OTU number (left) and relative abundance (right) of all kinds of bacteria in NRG and RG at different species classification levels. B. Phylum level of species. C. Family level of species. D. Genus level of species.

Figure 4

Different UM types and differential expression and correlation network of bacteria in two groups.

A. Composition of RG and NRG samples in each type. B. Types of UM samples which were visualized by PCoA and clustering at the genus level. C. Significant different bacteria enrichment between NRG and RG. The results could be biomarkers in RG. LDA score >2.0, $p < 0.05$. D. Co-occurrence networks of NRG and RG at the genus level. The size of the circle represents the abundance of bacteria. The thickness of the connected lines represents the strength of the relationship between bacteria.

Figure 5

Heatmap of bacteria and clinical characteristics. Cross axis listed clinical characteristics. The vertical axis showed different bacteria at the genus level. *: adjust $p \leq 0.05$. ***: adjust $p \leq 0.001$.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupFig1.tif](#)
- [SupFig2.tif](#)