Gut Microbiota is Associated with Response to 131I Therapy in Patients with Papillary Thyroid Carcinoma

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Abstract

Purpose

Radioactive Iodine (^{131}I) (RAI) therapy is a conventional post-surgery treatment widely used for papillary thyroid carcinoma (PTC). Since ^{131}I is orally administered, we hypothesize that it may affect gut microbiome. This study aims to investigate alterations of intestinal microbiome after ^{131}I therapy in PTC patients and explore its association with response to ^{131}I therapy.

Methods

Fecal samples of 60 PTC patients pre- and post-^{131}I therapy were collected to characterize the RAI-induced gut microbiota alterations using 16S rRNA gene sequencing. Sequence data of 40 out of the 60 patients, divided into excellent response (ER) group and non-excellent response (NER) group, were screened out to investigate the possible connection between gut microbiota and response to ^{131}I. A random forest classifier for ^{131}I response prediction was constructed.

Results

Microbial richness, diversity and composition were tremendously altered after ^{131}I therapy. A significant decline of Firmicutes to Bacteroides (F/B) ratio was observed post-^{131}I therapy. Some different metabolic pathways were found. Furthermore, discrepant β-diversity was found between the ER and NER groups pre- and post-^{131}I therapy. A predictive classifier for response to ^{131}I therapy with an AUC of 75.0% was constructed using four microbial variables-Bifidobacterium, Dorea, Erysipelotrichaceae, as biomarkers of ER and Parabacteroides, as biomarker of NER.

Conclusion

The present study illustrates the gut microbial dysbiosis after ^{131}I therapy in PTC patients for the first time and reveals a previously undefined role of gut microbiome as potential biomarkers for predicting RAI responses.

Introduction

Thyroid carcinoma (TC) is the commonest endocrine malignancy, the incidence of which is still increasing worldwide. Differentiated thyroid cancer (DTC) takes up over 90% of all TC, among which, papillary thyroid carcinoma (PTC) is the foremost histopathologic type, taking up more than 85% of TC(1).

RAI Therapy is the mainstay for the treatment of PTC after surgery(2), which has been used for thyroid cancer treatment for nearly 80 years and still plays a central role in the management of PTC till today(3). But response to ^{131}I therapy vary among PTC patients. Distinct responses to ^{131}I determine the following clinical strategies. According to the 2015 American thyroid association management guidelines for adult patients with thyroid nodules and differentiated thyroid cancer(4), responses to ^{131}I can be categorized as excellent response (ER) and non-excellent response (NER). ER to ^{131}I indicates clinical cure of PTC, whereas patients with NER to ^{131}I may require another course of radiotherapy. Hence, the need for a reliable tool predicting therapeutic response to ^{131}I ahead of radiotherapy is vital and urgent, but has not been fulfilled yet.

^{131}I is an orally administered radioactive nuclide used for internal-radiation therapy to treat PTC. After administration, ^{131}I stays and accumulates in the gastro-intestinal tract, thus is very likely to affect gut microbiome. Intriguingly, gut microbiota and its vital role in various diseases have drawn more and more attention. Accumulating evidences have indicated that gut microbiota plays a part in the pathophysiology of cancer(5). Recently, a close connection between gut flora and thyroid carcinoma has also been implicated(6). Gut microbiome is tremendously altered in thyroid carcinoma patients(7). Reciprocally, thyroid function is influenced by gut microbiota(8). On the other hand, gut microbiota is also reported to be associated with radiation sensitivity and radiation-related toxicities(9–11). Evidences from animal models have shown that gut microbiome composition may predict radiation injury(12).

However, the alterations of gut microbiota after ^{131}I therapy and its significance have not been investigated yet. Whether different gut microbiome is related to distinct responses to ^{131}I remains elusive. Can one or several gut genera be used as biomarkers to predict ^{131}I responses of PTC patients is still unknown.

Given the paucity of gut microbiome studies in ^{131}I treated PTC patients, we reported the present prospective study in a cohort of post operative PTC patients undergoing ^{131}I therapy. The present study demonstrated the impact of ^{131}I therapy on the gut microbiota, metabolic profile and compared the gut microbiota differences between patients with ER or NER to ^{131}I therapy both pre- and post- the therapy. Moreover, a response prediction random forest classifier of response to ^{131}I therapy was established and validated. Accordingly, our results shed new light on the predictive and therapeutic value of gut microbiota in treating PTC patients undergoing ^{131}I therapy.

Materials And Methods

Study participants

This study was performed in agreement with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of the First Affiliated Hospital of Third Military Medical University, China (Date Oct, 16th, 2020/No. KY2020214). The authors affirmed that all participants provided informed

consent for clinical data and bio-sample use and publication of their basic characteristics and 16s rRNA results of their stools.

All patients included in this study were from the southwest region of China (including Chongqing, Sichuan and Guizhou), where the climate and food habits were similar. $^{99m}TcO4^-$ thyroid imaging was performed before the ^{131}I therapy for every single participant, and no image of thyroid was observed. However, 48 hours after taking ^{131}I orally at a dose of 150mCi(5.55×10^9 Bq, a very small part of thyroid tissue was displayed in all of the patients by post RAI therapy whole-body scanning (RxWBS), with no obvious difference among them. Blood thyroid stimulating hormone (TSH) of each participant before ^{131}I therapy was over 30 mIU/L.

Self-controlled study cohort, recruitment of subjects, procedures of ^{131}I therapy and sampling

The inclusion criteria for TC patients were as follows: 1. Patients were diagnosed as PTC; 2. Patients had undergone complete thyroid resection procedure; 3. Patients were scheduled to receive ^{131}I radiotherapy for the first time; 4. Patients were willing to participate in this study and signed the informed consent forms; 5. Patients promised to voluntarily accept and comply with this experimental protocol.

The exclusion criteria were as follows: 1. Patients with known history of any other cancer; 2. Patients with prior ^{131}I therapy or other radiotherapy; 3. Patients with notable gastrointestinal disorder; 4. Patients with known history of gastro-intestinal surgery; 5. Patients with long-term use of any probiotic or antibiotic therapy, non-steroid anti-inflammatory drugs or proton pump inhibitors; 6. Patients with an age < 18 years. Finally, a total of 60 subjects from the nuclear medicine department of the First Affiliated Hospital of the Third Military Medical University, China, between October 2020 and March 2021, who fulfilled the inclusion criteria and provided the fecal and blood samples, were included in the self-controlled study for exploring microbiota changes induced by ^{131}I therapy.

Baseline characteristics and clinical parameters were listed in Table 1. Patients were given iodine-free diet for 4 weeks and undergone 3 weeks of Euthyrox withdrawal before each sampling. Two sequential peripheral blood and fecal samples were collected from patients at time points 1–2 days before and 5 months after ^{131}I administration respectively. ^{131}I was administered orally at a dose of 150 mCi. All samples were aliquoted and stored at -80°C for further use. TSH and Tg levels in the blood samples were assayed. DNA extraction and 16S rRNA gene sequencing were performed using the fecal samples.

Table 1
Demographic and clinical characteristics of participants in self-controlled study.

Baseline characteristics	Participants (n = 60)
Age, years: mean ± SD	40.02 ± 10.23
Gender: N(%)	Male: 19 (31.7%)
	Female: 41 (68.3%)
Risk level: N(%)	Low: 0 (0.0%)
	Medium: 49 (81.7%)
	High: 11 (18.3%)
Pathologic stage post-surgery: N(%)	□: 54 (90.0%)
	□: 4 (6.7%)
	□: 2 (3.3%)
	□: 0 (0.0%)
Maximum tumor diameter, cm: mean ± SD	1.38 ± 0.82
Local invasion by pathology: N(%)	Without: 43 (71.7%)
	With: 17 (28.3%)
Lymph node metastasis by RxWBS: N(%)	Without: 49 (81.7%)
	With: 11 (18.3%)
Distant metastasis by RxWBS: N(%)	Without: 59 (98.3%)
	With: 1 (1.7%)
TSH stimulated Tg level < 1 ng/ml: N(%)	25 (41.7%)
TSH stimulated Tg level: 1–10 ng/ml: N(%)	22 (36.7%)
TSH stimulated Tg level > 10 ng/ml or rising anti-Tg antibody levels: N(%)	13 (21.6%)
RxWBS, post RAI therapy whole-body scanning	

Patient demographics and clinical parameters of ^{131}I ER and NER patients

In order to investigate gut microbiota differences between PTC patients with distinct responses to ^{131}I therapy, overall, 20 of the 60 patients' data were eliminated due to exclusion criteria, which were Tg < 1 ng/ml even before ^{131}I therapy and no metastases observed by RxWBS or other imaging, indicating an excellent response to RAI therapy. The rest 40 participants were divided into ^{131}I ER group (24 cases) and ^{131}I NER group (16 cases).

The present study evaluated effectiveness of ^{131}I therapy according to these guidelines. After ^{131}I therapy, the patients who achieved excellent response were classified as excellent response group (ER), while patients whose responses belonged to the other categories were assorted as non-excellent response group (NER). Gut microbiota of the two groups both 1–2 days pre-treatment of ^{131}I (Pre-) and 5 months post-treatment (Post-) were analyzed and compared.

DNA extraction and 16S rRNA sequencing analysis

Fecal DNA extraction was performed using CTAB method. The V3-V4 region of the bacterial 16S rRNA gene was amplified by Phusion® High-Fidelity PCR Master Mix (New England Biolabs) using specific primers (515F-806R) with the barcode. PCR products purification was carried out with Qiagen Gel Extraction Kit (Qiagen, Germany). TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, USA) was used to generate Sequencing libraries. The libraries were sequenced using the Illumina NovaSeq 6000 platform of Novogene Company (Tianjin, China). We took advantage of Microbial Ecology 2 (QIIME2, version 2020. 2)(13) platform to process the sequencing data in a conda environment. In brief, the V3-V4 primers of paired-end fastq format sequence files were trimmed using cutadapt 3.1(14). Next, trimmed fastq files were imported into QIIME2. After the DADA2 denoising, a feature table listing sequence number of all samples and features was analyzed. Samples with less than 8000 sampling depth were excluded. All samples were normalized to the same depth. After rarefaction, alpha diversity and beta diversity were calculated respectively. Then, the calculation of the principal coordinate analysis (PcoA analysis) results was performed. Taxonomic composition of the samples was also displayed. The discrepantly abundant bacterial taxa between two groups were analyzed and displayed by linear discriminant analysis effect size (LEfSe) (15).

Establishment of response-prediction classifier for ^{131}I therapy

The random forest analysis using R package randomForest (16) was conducted to establish a response-prediction classifier to screen potential biomarkers predicting responses to ^{131}I from differentially abundant taxa found in LEfSe analysis pre-^{131}I therapy. The training cohort and the validation cohort were stratified random sampling with replacement selected at a 4:1 ratio from the pre-^{131}I therapy samples. After five repeats of seven-fold cross-validation to calibrate the model, followed by calculation of the best mtry (mtry = 4) to reduce the out-of-bag (OOB) error, finally, 3 genera and 1 family with high abundance selected from the differentially abundant microbes in the LEfSe analysis pre-^{131}I therapy were chosen as the optimal set of characteristic variables with the minimum error rate to establish a ^{131}I response prediction classifier, and verified in the validation cohort. The area under the operating characteristic curve (AUC) was employed to evaluate the performance of the classifier using R package pROC (17).

Bioinformatics and statistical analysis

Alpha (α) diversity was evaluated by a set of indexes, including Shannon, Simpson, ACE, CHAO1, etc., while beta (β) diversity was assessed using Bray-curtis distance-based non-metric multidimensional scaling (NMDS) analysis, supervised partial least squares-discriminant analysis (PLS-DA), principal coordinate analysis (PCoA) based on unweighted unifrac distance matrix or based on Jaccard index. Taxa bar plot and LEfSe analysis were performed to distinguish discrepant abundant genera between two groups. The predicted metabolic functional differences of gut microbiota between the two groups were compared using picrust2 to get differentially involved KEGG pathways.

The normality of distribution was determined using Shapiro-Wilk test. Homogeneity of variance was tested by F test. Comparison of baseline characters was performed by χ^2 or Student's t-test. Paired t test was used in the statistical analysis of self-controlled study. Significance between two groups was determined by Student's t-test or Wilcoxon-Rank test. *P* values < 0.05 were considered significant.

Results

Study cohorts and clinical parameters of self-controlled study

60 PTC patients post operation, who were planning for ^{131}I therapy, were included in the self-controlled study for exploring microbiota changes due to ^{131}I therapy. Baseline characters were listed in Table 1.

Microbial richness, diversity and composition alteration after ^{131}I therapy

From the 60 self-controlled samples, 7,540,436 high-quality sequences were used (average 59,845 per sample). The total number of ASV was 9,583 at 99% similarity level. The Good's coverage of each group was over 99% (Fig. 1a). In addition, alpha Rarefaction curve in each group is nearly smooth with a sufficient amount of sequencing data (Fig. 1b), indicating that the sequence depth represented the majority of gut bacteria in the samples.

The taxonomic α-diversities indicated by ACE (Abundance-based Coverage Estimator) richness (p = 8.6e − 05) and Chao1 (p = 0.00016) indexes displayed significantly lower community richness and diversity in the fecal microbiota after ^{131}I radiation therapy (Fig. 1c).

Shifts in β diversity (composition and structure) from pre-^{131}I therapy to post-^{131}I therapy were also observed on PCoA based on unweighted unifrac distance matrix or based on Jaccard index, NMDS analysis and PLS-DA (Fig. 1d). All results indicated that the samples within each group were clustered together, while the samples between groups were separated, illustrating prominent differences in bacterial structure pre-^{131}I therapy and post-^{131}I therapy (PCoA unweighted index: r = 0.2037 p = 0.001; PCoA Jaccard index: r = 0.2813 p = 0.001; NMDS: stress = 0.1366; PLS-DA: R2X = 0.128, R2Y = 0.733, Q2Y = 0.417).

Taxa bar plot showed the bacterial composition of each group in Phylum (Fig. 2a). The most prominent differences were decrease of Firmicutes and increase of Bacteroidetes composition post-^{131}I therapy

compared to pre-^{131}I therapy, consequently leading to a significant decline in the Firmicutes to Bacteroides (F/B) ratio (p = 0.0002) (Fig. 2b), suggesting dysbiosis following ^{131}I therapy. LEfSe analysis illustrated remarkably different microbes between the two groups with a LDA score over 3.5 (Fig. 2c), with notable increments in relative abundance of Bacteroidaceae and Prevotellaceae post-^{131}I therapy, and reverse trend in Ruminococcaceae on the family level. Genus-level distribution alterations of fecal microbiota was demonstrated by pie plot (Fig. 2d), showing an overall pattern of an increase in “pathogenic microbiota”, for example, Bacteroides, Prevotella_9, and a decrease in “beneficial microbiota”, including Roseburia, Blautia, etc.

Predicted Functional Changes of Microbiome induced by ^{131}I therapy

We further investigated the predicted functional alterations in gut microbiota owing to ^{131}I therapy. Analysis of KEGG pathways showed that compared with microbiome before ^{131}I therapy, after ^{131}I therapy, pathways involved in amino acid biosynthesis, biotin metabolism, secondary bile acid biosynthesis, one carbon pool by folate, other glycan degradation, lipopolysaccharide (LPS) biosynthesis etc. were markedly altered (Fig. 3).

Study cohorts and clinical parameters of ^{131}I ER and NER patients

After identifying the impact of ^{131}I therapy on gut microbiota, we then explored whether or not microbiome dysbiosis would correlate with therapeutic response to ^{131}I therapy. After aforementioned exclusion of 20 patients, 40 participants were included, and classified into ^{131}I ER group or NER group. Patients’ demographic features and clinical parameters were shown in Table 2.

Table 2
Demographic and clinical characteristics of participants in curative controlled-study

Baseline characteristics	Participants (n = 40)		Comparison between groups (*p* value)
	Excellent response (ER)	Non-excellent response (NER)	
Case: N(%)	24 (60%)	16 (40%)	
Age, years: mean ± SD	38.67 ± 9.7	35.63 ± 9.0	0.324
Gender: N(%)	Male: 8 (33.3%) Female: 16 (66.7%)	Male: 8 (50%) Female: 8 (50%)	0.234
Risk level: N(%)	Medium: 20 (83.3%) High: 4 (16.7%)	Medium: 14 (87.5%) High: 2 (12.5%)	0.718
pathologic stage post-surgery: N(%)	□: 22 (91.6) □: 1 (4.2%) □: 1 (4.2%)	□: 16 (100%) □: 0 (0%) □: 0 (0%)	1.000
Maximum tumor diameter, cm: mean ± SD	1.42 ± 0.95	1.48 ± 0.80	0.852
Local invasion by pathology: N(%)	Without: 18 (75%) With: 6 (25%)	13 (81.3%) 3 (18.7%)	0.717
Lymph node metastasis by RxWBS: N(%)	Without:15 (62.5%) With: 9 (37.5%)	14 (87.5%) 2 (12.5%)	0.148
Distant metastasis by RxWBS: N(%)	Without:23 (95.8%) With: 1 (4.2%)	16 (100%) 0 (0%)	1.000

RxWBS, post RAI therapy whole-body scanning

Baseline characteristics	Participants (n = 40)		Comparison between groups (*p* value)
	Excellent response (ER)	Non-excellent response (NER)	
TSH stimulated Tg level < 1 ng/ml: N(%)	4 (16.7%)	1 (6.3%)	0.186
TSH stimulated Tg level: 1–10 ng/ml: N(%)	15 (62.5%)	7 (43.7%)	
TSH stimulated Tg level > 10 ng/ml or rising anti-Tg antibody levels: N(%)	5 (20.8%)	8 (50%)	

RxWBS, post RAI therapy whole-body scanning

Gut microbiota richness, diversity and composition differences between ^{131}I ER and NER group pre- and post-^{131}I therapy

At 1–2 days prior to ^{131}I radiotherapy, from the 40 participants' samples, 2,268,985 high-quality sequences were used (average 56,725 per sample). The total number of ASV was 4,958 at 99% similarity level. The Good's coverage (over 99%) (Fig. 4a) and the observed species rarefaction curve (Fig. 4b) were used to characterize good sequencing depths. The microbiota of the two groups showed no significant difference in α-diversity (data not shown). β diversity analysis represented by PLS-DA-plot illustrated that the microbiome samples were clustered by group (Fig. 4c, R2X = 0.16, R2Y = 0.655, Q2Y = -0.179). Different bacterial composition of each group in Phylum was shown in taxa bar plot (Fig. 4d). A prominent decrease of F/B ratio was observed in the NER group as compared to ER group (Fig. 4e, p = 0.0118). LEfSe analysis illustrated substantially differently abundant bacterial genera, including Parabacteroides, Dorea, Bifidobacterium, etc. and microbial family, such as Erysipelotrichaceae, etc. with a LDA score over 2.0 between the two groups (Fig. 4f).

From the data of specimens collected at 5 months post-^{131}I radiotherapy, 2,253,269 high-quality sequences were used (average 60,832 per sample). The total number of ASV was 2,896 at 99% similarity level. The Good's coverage of each group was over 99% (Fig. 5a). Rarefaction curve indicated good sequencing depths (Fig. 5b). No significant α diversity was noted between ER and NER groups. however, in line with the result of pre-^{131}I therapy, a notable clustering effect of β-diversity by response status post-^{131}I was also revealed by PLS-DA-plot (Fig. 5c, R2X = 0.341, R2Y = 0.518, Q2Y = -0.083). Taxa bar plot showed elevation of Firmicutes and reduction of Bacteroidetes composition in the NER group (Fig. 5d), but no significant statistic difference was found in F/B ratio (Fig. 5e). LEfSe analysis illustrated significantly different bacterial taxa with a LDA score over 2.0 (Fig. 5f), including Erysipelotrichaceae family, etc.

Establishment of a predictive classifier for response to ^{131}I therapy on the gut microbiome profiles

Based on the results above, we speculated that the gut microbiome pre-^{131}I therapy might provide potential biomarkers for predicting response to ^{131}I therapy in post-surgery patients with PTC. To test this hypothesis, a random forest classifier for ^{131}I response prediction was constructed according to the taxonomic comparison analysis results pre-^{131}I therapy. Error rate was calculated according to mtry value in the random forest. The ntree value was set as 500 with a stable error rate lower than 0.092% (Fig. 6a). 9 potential discriminatory taxa, including 5 genera and 4 families got from LEfSe analysis (Fig. 4f), were used to form the set of input features. After five repeats of seven-fold cross-validation to calibrate the model, low abundance bacteria were filtered out. Four microbial variables, including Parabacteroides, Bifidobacterium, Dorea, Erysipelotrichaceae, were finally selected as the optimal set to construct the classifier in the training cohort, followed by verification in a validation cohort. In the classifier, composition of Parabacteroides and Bifidobacterium contributed the most to the MeaningDecreaseAccuracy and MeanDecreaseGini (Fig. 6b). The AUC was 75.0% in the validation cohort, suggesting that our microbiome-based classifier performed well on predicting response to ^{131}I therapy. Among the four microbial variables, Bifidobacterium, Dorea and Erysipelotrichaceae, were biomarkers of ER to ^{131}I therapy, whereas Parabacteroides, were biomarkers of NER to ^{131}I therapy.

Discussion

Previous literatures have shown variable results in gut microbiome alterations induced by irradiation exposure(11, 18–20). Nevertheless, the gut microbiota changes in the setting of ^{131}I therapy have not been elucidated yet. Coinciding with some other findings in gut microbiota changes post radiation(21), our data illustrated that ^{131}I therapy also resulted in deterioration of patients' microbiome alpha diversity and alteration of microbial composition, with an enrichment of "harmful microbiota" and a reduction in "beneficial microbiota" on the whole. The impact of ^{131}I therapy on gut microbiota was huge and durable, at least lasting until the end of our observation, 5 months post ^{131}I administration. As shown by accumulating literatures, gut microbiota dysbiosis is involved in the development of a wide range of diseases, including diarrhea, allergy, cancer, aging, diabetes, etc.(22), thus the dysbiosis after ^{131}I therapy described in the present study has great influence on intestinal homeostasis and PTC patients' health. Among the changes of gut microbiome, alteration in F/B ratio was worth noting.

In a healthy host, the majority of gut microbiome is typically dominated by four major phyla: Bacteroidetes, Firmicutes, Actinobacteria and Proteobacteria (23). Firmicutes and Bacteroidetes take up over 90% of the relative abundance of the gut microbiota and their relationship plays a critical role in the maintenance of gut homeostasis. Aberrant ratio between the relative abundance of Firmicutes and Bacteroidetes (F/B ratio) has been found in a series of physiological and pathological conditions, including aging(24), tumor(25), obesity(26), type □ diabetes(27), intestinal inflamm-ation, etc. In the present study, our data showed a prominent decline of Firmicutes post-^{131}I therapy, which is in accordance with Firmicutes change after pelvic radiotherapy(19), indicating a dysbiosis of gut microbiota

after RAI therapy. Of note, F/B ratio was also found to be declined in NER group compared to ER group, indicating a possible connection between response to ^{131}I therapy and microbial dysbiosis.

For the sake of understanding metabolic profile alterations of gut microbiota after ^{131}I therapy, KEGG analysis of stool samples was performed. Some of the increases in metabolites after ^{131}I therapy, such as bile acids and alanine, were in accordance with a previous reported characteristic elevation in radiation-induced acute intestinal symptoms in cervical cancer patients(28), indicating that metabolism of gut microbiome might share some common features post radiation, which warranted further study.

Previous literatures have shown that gut microbiota can offer a set of biomarkers with predictive value. In order to explore potential biomarkers for prediction of response to ^{131}I therapy, gut microbiome differences were compared between ^{131}I ER and NER groups both before and after the therapy. Different gut microbiota compositions were identified, which might act as a cause or as a result for the distinct responses to ^{131}I. There is also a possibility that some of the gut floras lead to different responses, and some others are the consequences of different responses. In agreement with a previous study investigating gut microbiota signatures of distinct responses to neoadjuvant chemoradiotherapy (18), Dorea, which is related with butyrate production, was found to be overrepresented in responders to ^{131}I pre-therapy as well, indicating some analogous characteristics of gut microbiota in response to radiation. It is of interest that Erysipelotrichaceae, which belongs to the Firmicutes phylum, with multiple interactions with host immune response, gut inflammation, and lipid metabolism, etc. (29), was markedly less abundant in patients with non-excellent response to ^{131}I therapy consistently before and after the therapy. Collectively, these results suggested that, as novel biomarkers, Dorea and Erysipelotrichaceae might not only provide targets for intervention, but also form the basis of establishing a response-prediction tool, which was later proven to be true in our predictive classifier. Unexpectedly, Lachnospiraceae family, identified to be positively associated with protection against radiation-induced intestinal damages (30), was found to be remarkably increased in the NER group post-^{131}I therapy, which awaits more study. The comparison of gut microbiota between groups with distinct responses to ^{131}I may provide additional information on how the gut microbiome interacts with radiotherapy responses.

Moreover, a predictive classifier for response to ^{131}I therapy on the gut microbiome profiles of PTC patients was established in this study. Four gut microbial variables, including Parabacteroides, Bifidobacterium, Dorea and Erysipelotrichaceae, for the first time, were identified to be valuable biomarkers capable of predicting response to ^{131}I therapy, which would be beneficial for customizing optimal therapeutic approaches for every PTC patient.

To sum up, the present study demonstrated gut microbiota changes after ^{131}I therapy in post-surgery PTC patients. Differences in gut flora were also found between ^{131}I ER and NER groups both pre- and post-^{131}I therapy, suggesting microbiome might be associated with therapeutic responses. Hence, a random forest classifier with several taxa as variables was established to provide a noninvasive tool for predicting response to ^{131}I therapy prior to treatment initiation.

These conclusions are based on analysis of data obtained from a small sample pool, reported in the pilot study herein. The long-term systemic effects of alterations in gut microbiome after ^{131}I therapy still need rigorous evaluation. With the aim to restore a healthy intestinal microbial ecosystem, which is beneficial for the response to ^{131}I therapy, personalized modulation of gut microbiota of PTC patients during the therapy, including dietary changes, administration of antibiotics or probiotics, fecal microorganism transfers, etc., is promising. Future studies with larger cohort of patients and employing whole genome metagenomics approaches may better define the optimal gut microbiome, which is the most beneficial for ^{131}I radiotherapy. Mice models of ^{131}I radiotherapy are needed to further explore the complicated association between gut microbiome and ^{131}I therapy and reveal the underlying mechanisms.

Declarations

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Competing Interests:

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions:

All authors contributed to the study conception and design. Material preparation and data collection were performed by Zheng L, Zhang LJ, Guo W, He S and Huang Y. Data were analyzed by Tang B, Xiao X, Tang L, Zheng L, Chen J, Huang DD, Pan D and Chen Y. The draft of the manuscript was written and revised by Chen J, Zheng L, Tang B and Xiao X. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics Approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of the First Affiliated Hospital of Third Military Medical University, China (Date Oct,16th,2020/No. KY2020214).

Consent to participate

Informed consent was obtained from all individual participants included in the study.

Consent to publish

The authors affirm that human research participants provided informed consent for publication of their data.

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Figures

Figure 1

Microbial richness and diversity alterations post-^{131}I therapy

Pre-^{131}I: 1-2 days before ^{131}I administration; Post-^{131}I: 5 months after ^{131}I administration. a: Goods_coverage indexes of two groups. b: Alpha rarefaction of each group indicating sequencing Depth. c: Ace and Chao1 indexes of the taxonomic α-diversities. ace: Paired t-test. P=8.6e-05. chao1: Paired t-test. *p*=0.00016. d: PCoA plot, NMDS and PLS-DA indexes of the β-diversity (Paired t-test, PCoA unweighted index: r = 0.2037 p = 0.001; PCoA Jaccard index: r = 0.2813 p = 0.001; NMDS: stress = 0.1366; PLS-DA: R2X = 0.128, R2Y = 0.733, Q2Y = 0.417).

Figure 2

Microbial composition alterations post-^{131}I therapy

a: Taxa bar plot illustrating the bacterial composition of each group in Phylum. b: Firmicutes to Bacteroides (F/B) ratio of the two groups. Paired t test. p=0.0002. c: Linear discriminant analysis effect size (LEfSe) analysis indicating differentially abundant bacterial genera with a LDA score over 3.5 between the two groups. d: Pie plot showing genus-level distribution of fecal microbiota.

Figure 3

Significantly altered KEGG pathways of gut microbiome post-^{131}I therapy

Figure 4

Significant differences of microbial richness, diversity and composition between ER and NER groups 1-2 days before ^{131}I therapy. ER: excellent response to ^{131}I therapy; NER: non-excellent response to ^{131}I therapy. a: Goods_coverage indexes of two groups. b: Alpha rarefaction of each group indicating sequencing Depth. c: PLS-DA-plot of the two groups. d: Taxa bar plot indicating the bacterial composition of each group in Phylum. e: Firmicutes to Bacteroides (F/B) ratio of the two groups. Paired t test. p=0.0002. f: LEfSe analysis showing significantly different gut flora with a LDA score over 2 between the two groups.

Figure 5

Significant differences of microbial richness, diversity and composition between 131I effective and ineffective groups 5 months after 131I therapy. a: Goods_coverage indexes of two groups. b: Alpha rarefaction of each group indicating sequencing Depth. c: Robbins and Singles indexes of the taxonomic α-diversities. d: PLS-DA-plot of the two groups. e: Taxa bar plot indicating the bacterial composition of each group in Phylum. f: LEfSe analysis showing significantly different gut flora with a LDA score over 2 between the two groups.

Figure 6

A predictive classifier for response to ^{131}I therapy on the gut microbiome profiles

a: Random forest model error and the ntree select of CART. b: Mean decrease accuracy and mean decrease gini of the random forest model. c: ROC curve of the random forest model.