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ORIGINAL RESEARCH

Prognostic Role of Short-Chain Fatty Acid-Producing Gut Microbiota and Gut Microbial Dynamics in Patients with Hepatocellular Carcinoma Receiving Chemoembolization: A Prospective Study

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Purpose: Transarterial chemoembolization (TACE) may cause gut dysbiosis by increasing portal vein pressure. However, its association with clinical outcomes remains unknown. We hypothesized that gut microbiota composition and diversity are associated with treatment response and prognosis in patients with hepatocellular carcinoma (HCC) undergoing TACE.

Patients and Methods: This single-center, prospective cohort study included 96 adult HCC patients treated with TACE from April 2021 to November 2023. Fecal samples were collected before TACE (P0), one day (P1), and one month (P2) after TACE. Fecal 16S rRNA taxonomy was analyzed to evaluate microbial diversity, composition, and dynamic changes at each time point. The primary outcome was the association between the initial response to TACE and changes in microbial diversity and composition.

Results: Out of the total participants, 63 (65.6%) were responders and 33 (34.4%) were non-responders. Responder stool samples had higher alpha-diversity than those of non-responders at baseline (median Shannon index: 4.26 vs 4.09), albeit not reaching statistical significance, and a higher abundance of short-chain fatty acid (SCFA)-producing bacteria at all time points. Alpha-diversity significantly decreased one day after TACE ($P < 0.05$ for P1 vs P0) and tended to recover one month later in the responders, albeit without statistical significance for P2 vs P0. Regarding beta-diversity, there were some changes in both responders and non-responders during the post-TACE period, albeit with different patterns. A low abundance of *Roseburia cecicola* (HR, 3.44; 95% CI, 1.10–10.8) and *Dialister_uc* (HR, 3.90; 95% CI, 1.32–11.6; both $P < 0.05$) at baseline was associated with worse overall survival.

Conclusion: Specific SCFA-producing bacteria, such as *Roseburia cecicola* and *Dialister_uc*, were associated with treatment response and survival after TACE in patients with HCC, suggesting a potential prognostic role of the gut microbiome.

Plain Language Summary:

- In this prospective study, the gut microbiomes of HCC patients who responded to TACE tended to have a higher baseline alpha-diversity than those of non-responders, with a significant difference in beta-diversity at baseline between the two groups.
- Alpha-diversity decreased immediately after TACE and tended to recover one month later, especially in patients achieving objective response who experienced relatively less prominent changes in beta-diversity.

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- Initial responders had more abundant short-chain fatty acid (SCFA)-producing bacteria at baseline than those of the non-responder group. SCFA-producing bacterial species *Roseburia cecicola* and *Dialister_uc* were dominant in the baseline stool of the responder group, whose abundance was significantly associated with improved survival outcomes after treatment.
- Initial responders and non-responders to TACE for HCC showed different gut microbial dynamics. Our study suggests that gut microbial diversity and specific microbial components could serve as potential predictors of clinical outcomes and as targets for adjunctive probiotic or postbiotic strategies for such patients.

Keywords: gut microbiome, hepatocellular carcinoma, transarterial chemoembolization, response, prognosis, short-chain fatty acid-producing bacteria

Introduction

The human gut microbiome plays a key role in intestinal inflammation and chronic inflammatory liver disease.[1] Disruption of the gut microbiome can trigger bacterial translocation and chronic inflammation of the liver, ultimately leading to hepatic fibrosis and hepatocellular carcinoma (HCC).[1–3] Although there have been many studies on the association between the gut microbiome and therapeutic outcomes in various solid tumors, studies focusing on HCC are limited.[4–7] Moreover, most existing studies have focused on the relationship between the gut microbiome and clinical responses to immunotherapy or fecal microbiome transplantation.[1,8]

In contrast, the role of gut microbiota in modulating outcomes after conventional locoregional therapies, such as transarterial chemoembolization (TACE), remains poorly understood. TACE is a treatment option for patients with a wide spectrum of unresectable HCC,[9,10] and combines the effects of conventional embolization and cytotoxic chemotherapy, enabling enhanced tumor drug delivery while minimizing systemic toxicity.[11] During chemoembolization, there may be a temporary rise in portal vein pressure, potentially leading to bacterial translocation and hepatic inflammation as the portal vein redirects intestinal blood flow to the liver.[11,12] Although one preclinical study reported microbiome changes after TACE in a rabbit model, it lacked a comparative analysis of post-TACE dynamics in cancer-bearing subjects.[13] To date, no clinical studies have investigated dynamic changes in gut microbiota in patients treated with TACE or their potential association with treatment response and survival.

We hypothesized that TACE-induced alterations in the gut microbiota may influence the clinical outcomes of HCC. Similar associations have been observed following immunotherapy, where short-chain fatty acid (SCFA)-producing bacteria correlate with therapeutic benefits by modulating the immune response and maintaining epithelial integrity. Characterizing microbiome dynamics after TACE may help identify prognostic taxa and novel host–microbiome interactions. Therefore, we conducted a prospective study to examine the association between dynamic changes in gut microbial composition and diversity and clinical outcomes in patients undergoing TACE for HCC. Additionally, we aimed to identify potential gut microorganisms related to therapeutic response and survival outcomes.

Material and Methods

Study Design and Variables

This was a single-center, prospective cohort study of participants with guidelines-based diagnosis of HCC[14,15] who were potential candidates for TACE between April 2021 and November 2023 at the Asan Medical Center, the highest-volume liver cancer center in Seoul, South Korea. Participants over 18 years old and eligible for TACE treatment at the initial decision-making stage were included in the study. If a participant had a history of previous TACE, the interval between their last TACE and the index date should be at least 6 weeks to allow for recovery of microbial diversity. Index dates were defined as the start dates of the first TACE procedure performed during the study period. Additionally, only participants who provided informed consent were enrolled. Participants who met any of the following criteria were deemed ineligible: (i) if they ultimately received treatment other than TACE, (ii) if baseline stool samples were not collected before TACE, (iii) if the amount of stool samples was insufficient for analysis, or (iv) if they withdrew their consent (Figure 1).

A total of 96 participants were enrolled. The end date of the follow-up period was May 31, 2024. Participant characteristics included demographic data, comorbidities, etiologies of HCC, and liver function at baseline. Additionally,

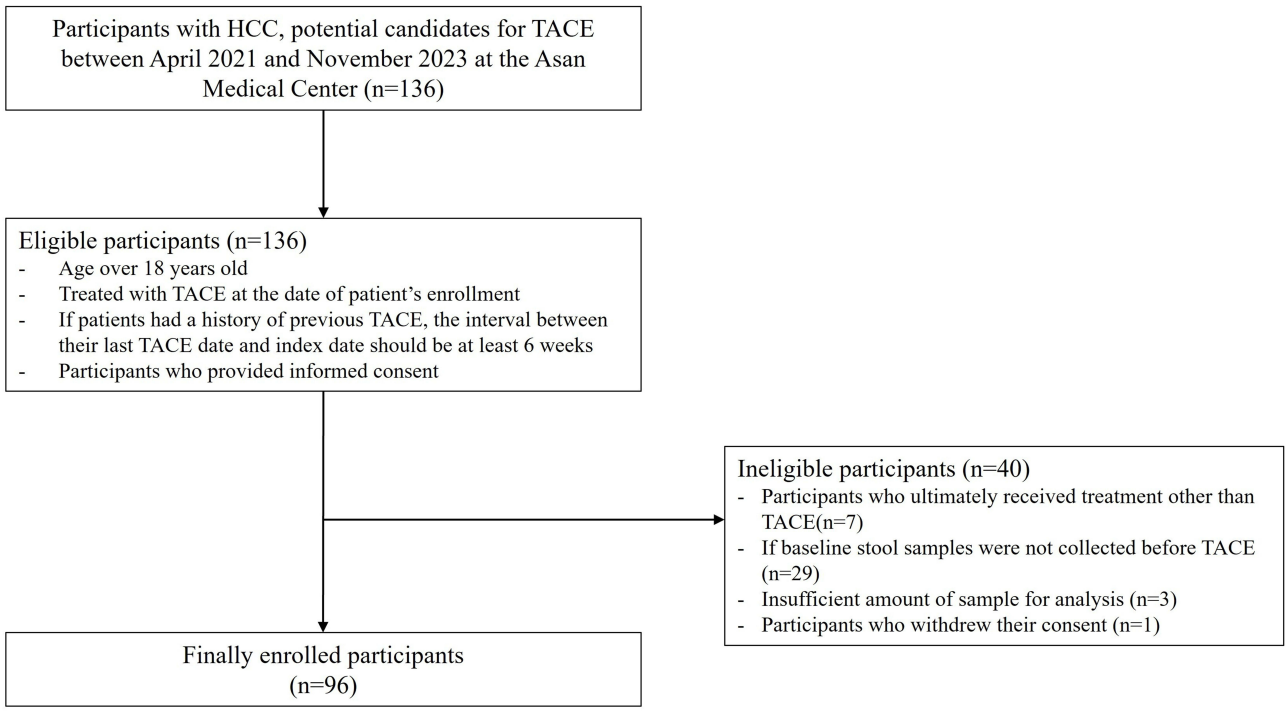

Figure 1 Study flow-chart.

data on baseline tumor characteristics were collected, including tumor markers, chemotherapeutic agents used in TACE, modified Union for International Cancer Control (mUICC) stage, and previous treatments before TACE. We further investigated participants' smoking history, alcohol consumption, use of analgesics, probiotics, multivitamins, herbal medicines, pre-treatment with proton pump inhibitors and antibiotics, and bowel habits.

This study was approved by the Institutional Review Board of Asan Medical Center (IRB approval no. 2021–0240) and was conducted in compliance with the ethical standards of the institutional research committee and the latest version of the Declaration of Helsinki. All participants provided written informed consent upon enrollment (Supplementary Material). This study adhered to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines for reporting observational studies (Table S1).

Transarterial Chemoembolization

All participants underwent TACE after the pre-treatment fecal samples had been collected. During TACE, following selective catheterization of the feeding artery, 50 mg of doxorubicin or 2 mg/kg of cisplatin was infused through the artery supplying the HCC. TACE was carried out as previously described.[16] All the participants received conventional TACE, and those who underwent drug-eluting bead TACE were not included in this study.

Collection of Fecal Samples, DNA Extraction, Bacterial 16S rRNA Sequencing, and Taxonomic Profiling

We obtained fecal samples within one day before TACE (P0), one day after (P1), and one month later (P2). While P0 fecal samples were collected from all participants, P1 and P2 samples were collected from 73 and 25 patients, respectively. The fecal samples were preserved using a DNA/RNA shield fecal collection kit and stored at –20°C. The detailed procedures for DNA extraction and 16S rRNA sequencing are described in the Supplementary Methods. The sequences obtained were analyzed in terms of Operational Taxonomic Units (OTU), and the species-level relative abundance was calculated as the proportion of the OTU counts attributed to each species on the phylogenetic tree.[17]

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Phylogenetic Diversity of the Gut Microbiome

OTU cluster analysis involved generating taxonomic composition charts for all taxonomic ranks from phylum to species. Alpha-diversity analysis (abundance-based coverage estimators [ACE], Chao, Shannon, and Simpson indices) and beta-diversity analysis (principal coordinate analysis) were conducted to assess bacterial diversity within the microbial communities.

Response Evaluation

Tumor response was initially assessed by multiphase computed tomography and/or liver magnetic resonance image one month after a single session of TACE. TACE was repeated every 6 to 8 weeks while viable HCC was present, with subsequent evaluations every 3 months for 2 years in the absence of viable HCC. After that, assessments were conducted every 6 months. For the evaluation of therapeutic response, we used modified Response Evaluation Criteria in Solid Tumors (mRECIST).[18] The responder was defined as a patient who achieved complete remission (CR) or partial remission (PR), whereas the non-responder was one who had stable disease (SD) or progressive disease (PD) at the first disease evaluation after TACE.

Study Outcomes

The primary outcome was the association between the initial treatment response to TACE and changes in microbial diversity and composition. The secondary outcome was the association between the altered gut microbiome and survival outcomes, such as overall survival (OS) and progression-free survival (PFS).

Subgroup Analysis Evaluating the Association Between Dynamic Changes in Gut Microbiome and Survival Outcomes

We conducted a linear discriminant analysis effect size (LEfSe) analysis with an effect size threshold of 3.0 or higher to identify significant differences in the relative abundances of bacterial taxa between the responder and non-responder groups. Patients were categorized into high-abundance and low-abundance subgroups based on median relative abundance ratios. Subsequently, we compared the OS and PFS of the high and low abundance subgroups with respect to selected bacterial species and identified the species showing statistically significant differences in survival outcomes between the two subgroups. Additionally, we analyzed the dynamic changes in diversity and relative abundance of these species after TACE in the responder and non-responder groups.

Statistical Analysis

Data are summarized as median with interquartile range (IQR) for continuous variables and numbers with percentages for categorical variables. Student's *t*-test or the Mann–Whitney *U*-test was used for continuous variables, and the chi-square or Fisher's exact test for categorical variables. Gut microbial diversity calculations and biomarker discovery programs were conducted by ChunLab Inc. (Seoul, South Korea) using in-house software.[19] For the analysis of bacterial diversity, alpha diversity indexes were calculated using the R program package "vegan". Unweighted and weighted Unifrac distances for evaluating beta-diversity were calculated with the phyloseq package. Taxonomic biomarkers and functional biomarkers were identified through statistical comparison algorithms (LEfSe and Kruskal–Wallis *H*-Test) using functional profiles predicted by PICRUS[20] and MinPath algorithms.[21] All analyses were performed in EzBioCloud, a 16S-based microbial taxonomic profiling platform developed by ChunLab.[22]

OS and PFS were estimated using the Kaplan-Meier method and compared by the Log rank test. Cox regression analysis was used to identify the risk factors associated with OS and PFS. Statistical analyses were performed using R software (http://cran.r-project.org/). *P*-values of less than 0.05 were considered statistically significant.

Results

Baseline Characteristics and Treatment Responses After TACE

The median age of the entire cohort was 65.0 (59.0–72.0) years. Most patients were male (84.4%) and had chronic hepatitis B (60.4%) as the cause of HCC (Table 1). At baseline, 51 patients (53.1%) had preserved liver function, corresponding to Child-Pugh class A, and 83 patients (86.5%) had liver cirrhosis. Over half of the patients were classified

Table 1 Baseline Characteristics of Study Population

Variable	Entire Cohort (n=96)	Responders (n=63)	Non-Responders (n=33)	*P*
Male sex	81 (84.4)	55 (87.3)	26 (78.8)	0.43
Age, years	65.0 (59.0–72.0)	65.0 (59.0–72.0)	66.0 (61.0–71.0)	0.86
BMI, kg/m^2	25.5 (22.4–27.7)	25.6 (22.4–27.4)	25.5 (22.5–28.0)	0.98
Family history of HCC				0.61
None	77 (80.2)	49 (77.8)	28 (84.8)	
Present	18 (18.8)	13 (20.6)	5 (15.2)	
Unknown	1 (1.0)	1 (1.6)	0 (0.0)	
Hypertension	40 (41.7)	27 (42.9)	13 (39.4)	0.91
Diabetes	36 (37.5)	24 (38.1)	12 (36.4)	0.99
Fatty liver	22 (22.9)	14 (22.2)	8 (24.2)	0.99
Alcohol consumption				0.27
None/Ex-drinker	61 (63.6)	43 (68.3)	18 (64.5)	
Current drinker	35 (36.4)	20 (31.7)	15 (45.5)	
Smoking history				0.93
None/Ex-smoker	66 (68.8)	44 (69.8)	22 (66.7)	
Current smoker	30 (31.2)	19 (30.2)	11 (33.3)	
Etiology of HCC				0.28
Hepatitis B	58 (60.4)	38 (60.3)	20 (60.6)	
Hepatitis C	7 (7.3)	6 (9.5)	1 (3.0)	
Alcohol	21 (21.9)	11 (17.5)	10 (30.3)	
Others	10 (10.4)	8 (12.7)	2 (6.1)	
Child-Pugh class				0.03
A	51 (53.1)	39 (61.9)	12 (36.4)	
B	45 (46.9)	24 (38.1)	21 (63.6)	
ALBI	−1.9 (−2.1, −1.5)	−1.9 (−2.2, −1.6)	1.8 (−2.1, −1.3)	0.04
ALBI grade				0.09
Grade 1	1 (1.0)	1 (1.6)	0 (0.0)	
Grade 2	77 (80.2)	54 (85.7)	23 (69.7)	
Grade 3	18 (18.8)	8 (12.7)	10 (30.3)	
Cirrhosis	83 (86.5)	52 (82.5)	31 (93.9)	0.22
AFP < 200 ng/mL	76 (79.2)	51 (81.0)	25 (75.8)	0.74
AFP ≥ 200 ng/mL	20 (20.8)	12 (19.0)	8 (24.2)	
Ascites				0.20
None	83 (86.5)	57 (90.5)	26 (78.8)	
Present	13 (13.5)	6 (9.5)	7 (21.1)	
Number of previous TACE sessions	Range: 0–9	Range: 0–8	Range: 0–9	0.53
Chemotherapeutic agent				0.24
Cisplatin	75 (78.1)	52 (82.5)	23 (69.7)	
Adriamycin	21 (21.9)	11 (17.5)	10 (30.3)	
mUICC stage				0.77
I–II	46 (47.9)	29 (46.0)	17 (51.5)	
III–IV	50 (52.1)	34 (54.0)	16 (48.5)	

(*Continued*)

Table 1 (Continued).

Variable	Entire Cohort (n=96)	Responders (n=63)	Non-Responders (n=33)	*P*
Previous treatment	27 (28.1)	18 (28.6)	9 (27.3)	0.99
Surgery	7 (25.9)	6 (33.3)	1 (11.1)	
TACE[a]	27 (100.0)	17 (94.4)	8 (88.9)	
RFA	5 (18.5)	3 (16.7)	2 (22.2)	
RT	6 (22.2)	3 (16.7)	3 (33.3)	
Systemic chemotherapy	1 (3.7)	0 (0.0)	1 (11.1)	
Bristol type				0.21
Type 1–2	5 (5.2)	2 (3.2)	3 (9.1)	
Type 3–4	72 (75.0)	49 (77.8)	23 (69.7)	
Type 5–7	19 (19.8)	12 (19.0)	7 (21.2)	
Pre-medication history	74 (77.1)	47 (74.6)	27 (81.8)	0.59
Probiotics	44 (59.5)	29 (61.7)	15 (55.6)	
Analgesics	28 (37.8)	14 (29.8)	14 (51.9)	
Herbal medication	30 (40.5)	22 (46.8)	8 (29.6)	
Multivitamin complex	40 (54.1)	22 (36.8)	18 (66.7)	
Proton pump inhibitors	33 (44.6)	18 (38.3)	15 (55.6)	
Antibiotics	21 (28.4)	11 (23.4)	10 (37.0)	

Notes: Data are presented as n (%) or median (interquartile range). [a]The most recent TACE was performed more than 6 weeks before the index date to allow for sufficient recovery of liver function and gut microbial diversity, as referenced in Bian et al.[13]
Abbreviations: ALBI, albumin-bilirubin index; AFP, alpha-fetoprotein; BMI, body mass index; HCC, hepatocellular carcinoma; mUICC, modified Union for International Cancer Control; RFA, radiofrequency ablation; RT, radiotherapy; TACE, transarterial chemoembolization.

as mUICC stage III or IV (52.1%). The number of previous sessions of TACE before the index date ranged from 0 to 9. Before initiating TACE, 27 (28.1%) patients had received other previous treatments such as hepatic resection, radiofrequency ablation, radiotherapy, and systemic chemotherapy.

When initial treatment responses after TACE were classified according to mRECIST criteria, 63 patients (65.6%) were classified as responders (CR, 26 patients [27.1%]; and PR, 37 patients [38.5%]), while 33 patients were non-responders (SD, 27 patients [28.1%]; and PD, 6 patients [6.3%]) (Table 2). The objective response rate and disease control rate at the initial response were 65.6% and 93.7%, respectively. There were no significant differences in patient preference factors such as smoking history, alcohol consumption, and bowel habits between initial responders and non-

Table 2 Initial Response to TACE Based on Modified RECIST in the Entire Cohort (n = 96)

Initial Response	n (%)
Complete remission	26 (27.1)
Partial response	37 (38.5)
Stable disease	27 (28.1)
Progressive disease	6 (6.3)
Objective response rate[a]	65.6%
Disease control rate[b]	93.7%

Notes: Data are presented as n (%). [a]Objective response rate is defined as the proportion of all patients with complete remission or partial remission. [b]Disease control rate is defined as the proportion of all patients with complete remission, partial remission, or stable disease.
Abbreviations: RECIST, Response Evaluation Criteria in Solid Tumors; TACE, transarterial chemoembolization.

responders. Furthermore, there were no significant differences between the two groups regarding pre-medication including probiotics, proton pump inhibitors and antibiotics (Table 1).

Phylogenetic Diversity of the Gut Microbiome at Baseline in Initial Responders and Non-Responders

To investigate whether the response to TACE treatment is associated with gut dysbiosis, gut metagenome profiles in the baseline stool samples (P0) were examined in 63 responders and 33 non-responders. Alpha- and beta-diversity values were compared to evaluate the microbial diversity in these two groups. Alpha-diversity was assessed by calculating the ACE, Chao1, Shannon, and Simpson indices (Figure 2A). While there were no significant differences in any of these indices, the median alpha-diversity was higher in the responder group than in the non-responder group (Shannon index: 4.26 vs 4.09). When beta-diversity within these two groups was analyzed using the Bray-Curtis distance, a notable distinction in bacterial community composition was observed between the two groups ($P < 0.001$; Figure 2B), suggesting a clear dissimilarity in the microbial community between the groups at baseline.

Baseline Gut Microbial Composition and Differentially Abundant Taxa at the Genus and Species Levels in Initial Responders and Non-Responders

The presence of bacterial taxa in stool, which could potentially differentiate between the gut microbiota of the two groups, was assessed. At the phylum level, no distinct differences were observed in the bacterial profiles of the responder and non-responder groups. Firmicutes dominated the microbiota in both groups, followed by Bacteroidetes, Proteobacteria, Actinobacteriota, and Fusobacteriota (Figure 2C). At the family level, Prevoteallaceae and Ruminococcaceae were more abundant in the responders than in the non-responders, whereas Lachnospiraceae and Bacteroidaceae were less abundant (Figure 2D). At the genus level, the responder group exhibited a higher abundance of *Prevotella* and *Faecalibacterium* compared to the non-responder group, and a lower abundance of *Bacteroides* (Figure 2E).

The phylogenetic distribution of differentially abundant taxa at the genus and species levels was assessed using a cladogram and the plot of the Linear Discriminant Analysis (LDA) score (Figure S1). At the genus level, *Roseburia*, PAC001138_g, PAC001046_g, and PAC001043_g were dominant in the responder group, whereas *Peptostreptococcaceae* was dominant in the non-responder group (Figure S1A and B). At the species level with an LDA score ≥ 3.0, *Roseburia cecicola* group, *Dialister_uc*, PAC001046_s, and DQ797020_s were identified as responder-dominant species in the baseline stool, whereas *Ruminococcus gnavus* was a non-responder dominant species (Figure S1B). Among them, the greater abundance of *Roseburia cecicola, Dialister_uc*, and DQ797020_s in the responder group was statistically significant (all $P < 0.01$, Figure S2).

Changes in Alpha- and Beta-Diversity of the Gut Microbiome From Baseline to One month After TACE

Alpha-diversity was compared to assess changes in the richness and evenness of microbiomes over the post-TACE period in the responder and non-responder groups (Figure S3). Microbial results in stool at P1 and P2 were available from 46 and 15 responders, and 27 and 10 non-responders, respectively. In the responder group, bacterial richness and evenness were significantly reduced the day after TACE (P1) ($Ps < 0.05$ for all alpha-diversity indices) but showed a tendency to recover after a month (P2) ($Ps < 0.001$ for P2 vs P1 in ACE and Chao1, Figure S3A). Similar alpha-diversity patterns were observed in the non-responder group, albeit not statistically significant (Figure S3B). Over the post-TACE period from P0 to P2, there were some temporal changes of beta-diversity at the genus level in both responders and non-responders. However, the non-responder group displayed relatively greater changes and variability in microbial composition after TACE (Figure S4).

Changes in the Relative Abundance of Responder-Dominant Species During the Post-TACE Period Based on the Initial Treatment Response

The relative abundance of *Roseburia cecicola* and PAC001046_s, identified as responder-dominant species, decreased significantly the day after TACE ($P = 0.008$ and $P = 0.001$, respectively for P1 vs P0) and remained low until one month

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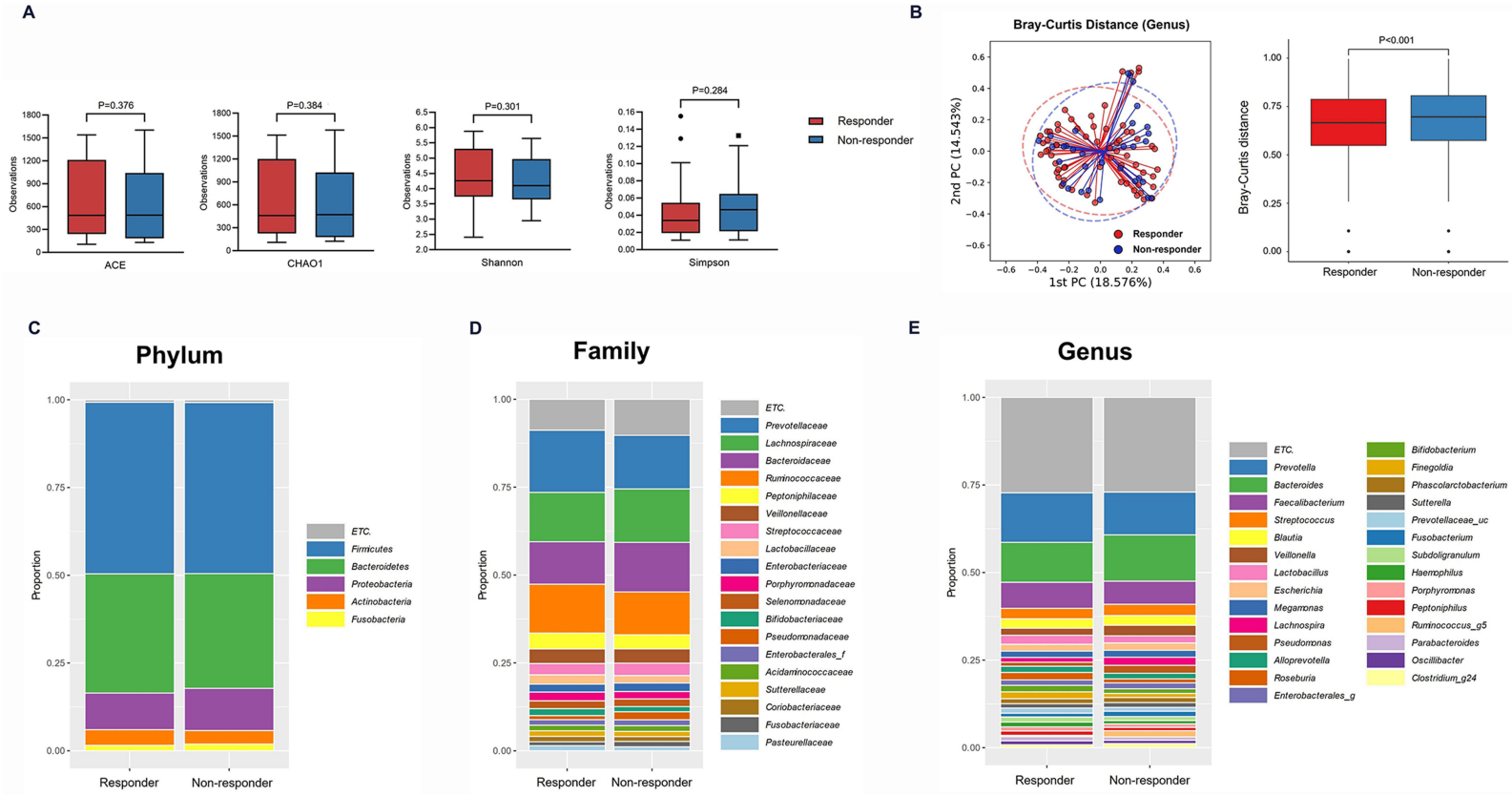

Figure 2 Comparison of baseline microbial diversity and relative abundance of the gut microbiome in the responder and non-responder groups. (**A**) Baseline alpha-diversity. (**B**) Baseline beta-diversity. (**C**) Relative abundance of the baseline gut microbiome at the phylum level. (**D**) Relative abundance of the baseline gut microbiome at the family level. (**E**) Relative abundance of the baseline gut microbiome at the genus level.

later in the responder group ($P = 0.514$ and $P = 0.234$, respectively for P2 vs P1; Figure S5A). In terms of *Dialister_uc*, the relative abundance also decreased over time after TACE ($P = 0.019$ for P2 vs P0 and $P = 0.043$ for P2 vs P1; Figure S5A). In the non-responder group, a similar pattern of relative abundance was observed only in PAC001046_s ($P = 0.013$ for P1 vs P0 and $P = 0.397$ for P2 vs P1; Figure S5B).

Association Between Responder-Dominant Species and Survival Outcomes After TACE

During a median follow-up duration of 6.8 months (IQR, 2.1–9.7 months), 16 patients (16.7%) died and 46 patients (47.9%) experienced tumor progression according to mRECIST (Figure S6A and B). The overall median OS and PFS were 23 (95% CI, 13.7–Not estimable) months and 7.0 (95% CI, 6.0–12.1) months, respectively. The OS rates at 5 months, 10 months, 15 months, and 25 months were 94.9%, 82.6%, 63.1%, and 39.4%, respectively, while the PFS rates were 69.9%, 34.0%, 21.6%, and 11.6%, respectively.

The abundance of *Roseburia cecicola* and *Dialister_uc* in baseline stool was significantly associated with survival outcomes. The subgroups with high abundance of either species had significantly better OS than the corresponding low abundance subgroups (*Ps* < 0.05 by Log rank test for both; Figure 3A and C), with cut-offs for abundance of 0.005 (*Roseburia cecicola*) and 8.0×10^{-5} (*Dialister_uc*) based on median relative abundance ratios. Regarding *Roseburia cecicola*, the low abundance subgroup had a significantly worse OS than the high abundance subgroup (median OS, 13.7

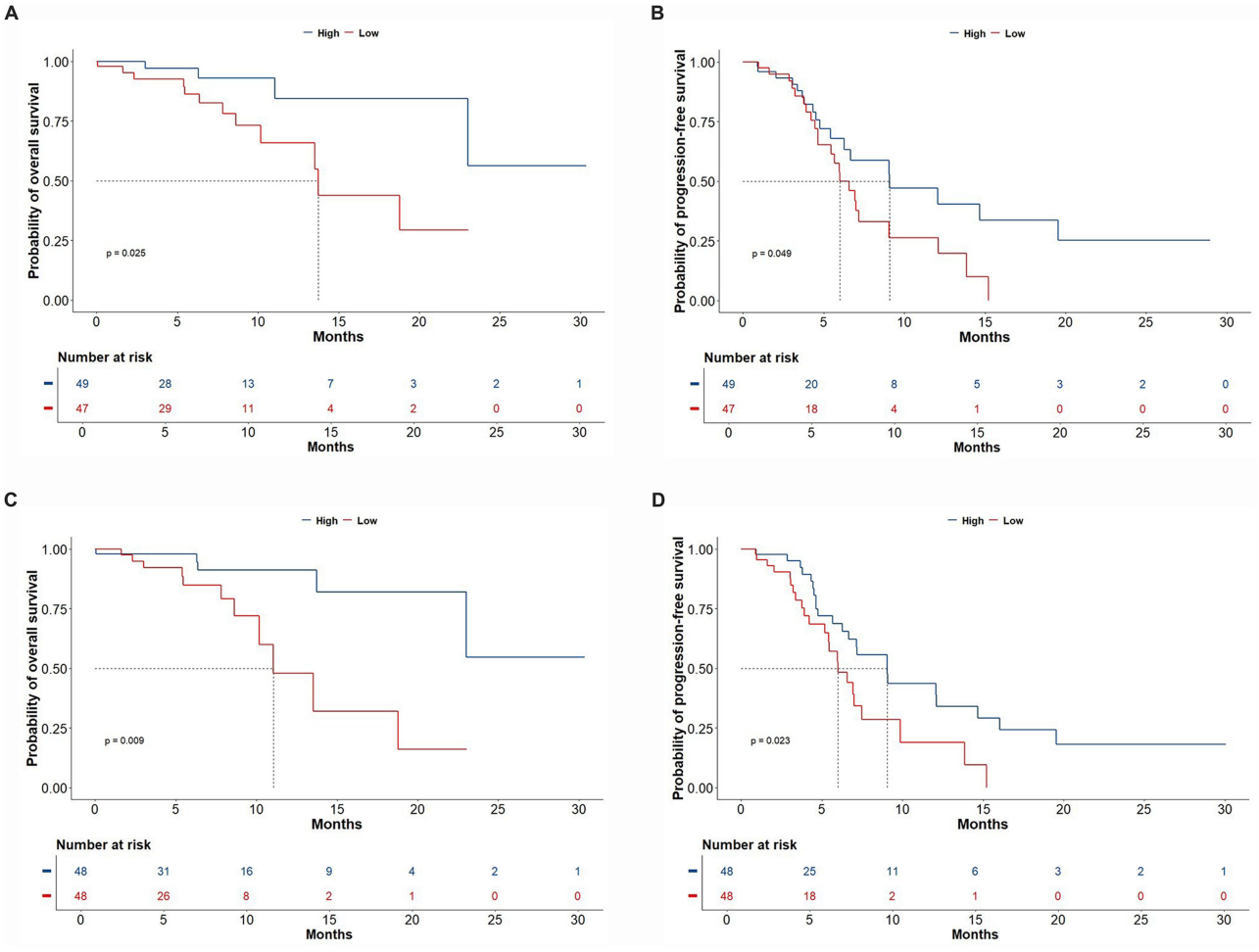

Figure 3 Overall survival and progression-free survival according to the abundance of *Roseburia cecicola* and *Dialister_uc*. (**A**) Overall survival according to the abundance of *Roseburia cecicola*. (**B**) Progression-free survival according to the abundance of *Roseburia cecicola*. (**C**) Overall survival according to the abundance of *Dialister_uc*. (**D**) Progression-free survival according to the abundance of *Dialister_uc*.

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months vs not reached; $P = 0.025$; Figure 3A). The same finding was found for *Dialister_uc* with a median OS of 11.1 months vs not reached (P=0.009; Figure 3C). For both *Roseburia cecicola* and *Dialister_uc*, there were significant differences in PFS between the low vs the high abundance groups (median PFS, 6.6 months vs 9.1 months; $P = 0.049$ for *Roseburia cecicola*; and 6.0 months vs 9.1 months; $P = 0.023$ for *Dialister_uc*; Figure 3B and D). However, neither patient-related nor tumor-related factors at baseline were significantly different between low- and high-abundance subgroups of both species (Table S2).

In the Cox regression analysis for identifying risk factors of OS, low abundances of *Roseburia cecicola* (HR, 3.44; 95% CI, 1.10–10.8; $P = 0.03$) and *Dialister_uc* (HR, 3.90; 95% CI, 1.32–11.6; $P = 0.01$) at baseline were the only factors significantly associated with worse OS (Table 3). Regarding risk factors associated with PFS, a low abundance of

Table 3 Univariate and Multivariable Analysis on Factors Associated with Overall Survival and Progression Free Survival in Entire Cohort

Variables	Overall Survival			Progression-Free Survival					
	Univariate Analysis			Univariate Analysis			Multivariable Analysis		
	HR	95% CI	*P*	HR	95% CI	*P*	aHR	95% CI	*P*
Male Sex	0.60	0.13–2.86	0.50	0.56	0.25–1.24	0.20	–	–	–
Age									
< 60 years	1	Reference	0.30	1	Reference	0.08	–	–	–
≥ 60 years	1.98	0.56–7.00		1.90	0.94–3.87				
BMI									
< 23 kg/m^2	1	Reference	0.20	1	Reference	>0.99	–	–	–
≥ 23 kg/m^2	0.54	0.19–1.50		0.97	0.50–1.86				
Diabetes, presence	1.00	0.36–2.78	>0.99	1.20	0.63–2.26	0.60	–	–	–
Etiology of HCC									
Non-viral	1	Reference	0.60	1	Reference	0.70	–	–	–
Viral	0.78	0.27–2.25		0.90	0.47–1.71				
Child Pugh class									
A	1	Reference	0.40	1	Reference	0.50	–	–	–
B	1.58	0.54–4.63		0.81	0.45–1.46				
Smoking, presence	0.63	0.21–1.87	0.40	1.87	0.90–3.89	0.09	–	–	–
Alcohol, presence	1.66	0.47–5.85	0.40	1.79	0.86–3.71	0.12	–	–	–
Cirrhosis	0.54	0.07–4.36	0.60	0.22	0.08–0.59	0.003	0.22	0.08–0.61	0.004
mUICC stage									
I–II	1	Reference	0.13	1	Reference	0.01	1	Reference	0.01
III–IV	2.44	0.76–7.81		2.41	1.28–4.54		2.26	1.19–4.30	
Roseburia cecicola							–	–	–
High abundance	1	Reference	0.03	1	Reference	0.05			
Low abundance	3.44	1.10–10.8		1.81	1.00–3.31				
Dialister_uc									
High abundance	1	Reference	0.01	1	Reference	0.03	1	Reference	0.01
Low abundance	3.90	1.32–11.6		2.00	1.09–3.67		2.23	1.20–4.11	
Ruminococcus gnavus									
High abundance	1	Reference	0.50	1	Reference	0.3	–	–	–
Low abundance	1.42	0.53–3.84		0.74	0.41–1.34				

Abbreviations: AFP, alpha-fetoprotein; aHR, adjusted hazard ratio; BMI, body mass index; CI, confidence interval; HCC, hepatocellular carcinoma; HR, hazard ratio; mUICC, modified Union for International Cancer Control.

Dialister_uc (HR, 2.00; 95% CI, 1.09–3.67; $P = 0.03$), together with mUICC stage III or IV (HR, 2.41; 95% CI, 1.28–4.54; $P = 0.01$), was significantly associated with worse PFS in the univariate analysis. A low abundance of *Roseburia cecicola* was also identified as a potential risk factor for worse PFS (HR, 1.81; 95% CI, 1.00–3.31; $P = 0.05$). However, in the multivariable analysis, only a low abundance of *Dialister_uc*, not *Roseburia cecicola*, remained significantly associated with worse PFS (adjusted HR, 2.23; 95% CI, 1.20–4.11; $P = 0.01$).

Discussion

In this prospective study, the gut microbiomes of initial responders tended to have a higher baseline alpha-diversity than those of non-responders, with a significant difference in beta-diversity at baseline between the two groups. Alpha-diversity decreased immediately after TACE and tended to recover one month later, more notably in patients achieving objective response who experienced relatively less prominent changes in beta-diversity. At baseline, the gut microbiome of the responder group had more abundant short-chain fatty acid (SCFA)-producing bacterial genera such as Prevotella and Faecalibacterium than that of the non-responder group. As SCFA-producing bacterial species, *Roseburia cecicola* and *Dialister_uc* were also dominant in the baseline stool of responders. Particularly, a high abundance of the two species before TACE was significantly associated with improved survival outcomes after treatment.

TACE has a transient effect on hepatic hemodynamics by decreasing hepatic arterial blood flow and increasing portal blood pressure.[11,12] This physiological mechanism leads to bowel wall edema and impaired intestinal barrier function, resulting in the disruption of the gut-liver signaling axis. In a preclinical study by *Bian* et al[13] alpha-diversity of the gut microbiome decreased immediately after TACE and then recovered over a short period, which was consistent with our findings from clinical patients with HCC. Conversely, the SCFA-producing bacteria have been reported to have a positive effect on the therapeutic response to immunotherapy in several cancer types.[6,23–25] Interestingly, *Dialister* and *Roseburia*, specific microbial species identified to be closely associated with the initial response and long-term survivals in our TACE series, were also abundant in the microbiomes of responders with HCC treated with immunotherapy.[23,26] Butyrate and propionate are SCFAs produced by the *Dialister* and *Roseburia* species, which have been found to inhibit tumor cell growth and promote apoptosis in various tumors including liver cancers.[23,27,28] Such anti-cancer effects of beneficial metabolic products from specific microbiota could likely contribute to enhancing TACE response in patients with HCC.

However, the abundance of both *Roseburia cecicola* and *Dialister_uc* seemed to decrease with time following TACE in our observations. This might result in intestinal homeostatic imbalance through reshaping gut microbiota structure due to increased intestinal permeability and immune activity linked to transient portal hypertension after TACE. Therefore, microbiota-based control of intestinal homeostasis by postbiotics, including SCFAs, could represent a novel therapeutic adjunct in patients with potentially anticipated TACE refractoriness.[29,30] Conversely, there is growing interest in the development of non-invasive biomarkers, such as radiomics-based models that evaluate the tumor immune microenvironment to predict treatment response in HCC.[31] In a similar context, our findings suggest that microbiome profiling may serve as a non-invasive tool for predicting clinical prognosis in patients with HCC treated with TACE.

To the best of our knowledge, our study is the first prospective cohort study to investigate the association between intestinal microbiota composition and clinical outcomes after TACE. However, there were some limitations. First, the dietary habits of the study population could not be strictly controlled. However, most patients followed a traditional Korean-style balanced diet[32] at enrollment. Considering the significant impact of diet on the gut microbiome, future studies should adjust for the type of diet to better understand its influence. Second, the single-center design and limited sample size may restrict the generalizability of our findings to a broader population. In addition, the lack of available P2 stool samples for a substantial portion of patients probably limited our ability to detect statistically significant associations. Achieving statistical significance requires an adequate sample size; however, as no previous studies have evaluated gut microbial changes in relation to prognosis in patients with TACE-treated HCC, a formal sample size calculation could not be performed. Therefore, future studies with larger cohorts are required to validate and expand these preliminary findings. Third, we did not perform direct functional validation of the gut microbiota, such as quantitative measurements of SCFA metabolites or mechanistic experiments to confirm the causality. Although we observed associations between specific microbial taxa and clinical outcomes, the underlying biological mechanisms remain to be fully elucidated. Furthermore, while 16S rRNA sequencing is widely used for characterizing the complexity of microbial communities, it

has inherent limitations in taxonomic resolution, as closely related species may be indistinguishable due to sequence homology.[33] Therefore, future studies incorporating functional analyses, such as SCFA quantification using gas chromatography, mass spectrometry, and genome-level microbial characterization via shotgun metagenomic sequencing, are warranted to elucidate causal pathways more precisely. In addition, in vitro experiments, such as the inhibition of SCFA production in responder-derived microbiota or supplementation of synthetic SCFAs to non-responder microbiota, followed by evaluation of TACE efficacy in HCC models, may provide further mechanistic insight into the causal role of SCFAs. Fourth, we did not evaluate the potential impact of cytotoxic agents, such as adriamycin and cisplatin, on the gut microbiome. The gut microbiome is also influenced by cytotoxic chemotherapy. However, as most patients (78.1%) received a cisplatin-containing regimen, the variation in clinical outcomes attributable to differences in chemotherapeutic agents is likely to be minimal.

Conclusion

In conclusion, initial responders to TACE tended to have higher alpha-diversity at baseline and exhibited relatively smaller peri-treatment changes in beta-diversity compared to non-responders. A higher relative abundance of SCFA-producing bacteria, such as *Roseburia cecicola* and *Dialister_uc*, prior to TACE was also observed in responders and appeared to be associated with favorable survival outcomes. Although these findings suggest a potential link between gut microbiota and treatment response, further randomized studies are required to investigate the potential role of microbiome-targeted interventions, such as postbiotics, in patients undergoing TACE.

Abbreviations

ACE, Abundance-based Coverage Estimator; CI, confidence interval; CR, complete remission; HCC, hepatocellular carcinoma; HR, hazard ratio; IQR, interquartile range; LEfSe, linear discriminant analysis effect size; LDA, Linear Discriminant Analysis; mRECIST, modified Response Evaluation Criteria in Solid Tumors; mUICC, modified Union for International Cancer Control; OS, overall survival; OTU, Operational Taxonomic Units; PD, progressive disease; PFS, progression-free survival; PR, partial remission; SCFA, short chain fatty acid; SD, stable disease; TACE, transarterial chemoembolization.

Data Sharing Statement

All data generated or analyzed in this study are included in this article. Further inquiries should be directed to the corresponding author, Professor Ju Hyun Shim.

Statement of Ethics

Written informed consent was obtained from all participants in this study. This study protocol was reviewed and approved by the Institutional Review Board of Asan Medical Center, approval number (IRB approval No. 2021-0240).

Author Contributions

All authors met the authorship criteria. JH Shim and JY Jeong are guarantors of the article. All authors made substantial contributions to the conception and design of the study, acquisition, analysis, or interpretation of data, and drafting, critical revision, and final approval of the manuscript. J Yang, J Lim, JY Jeong, and JH Shim were primarily responsible for study design, data analysis, statistical interpretation, and manuscript drafting. EH Kim, J An, D Lee, and HC Lee contributed to data acquisition and critical revision of the manuscript. All authors reviewed and approved the final version of the manuscript, agreed on the journal of submission, and accepted responsibility for the integrity and accuracy of the study.

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Disclosure

The authors report no conflicts of interest in this work.

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