



Microbiota-metabolite signatures in metastatic colorectal cancer: Promise, pitfalls, and the path forward

Ting-Ting Zhang, Juan Yao, Han-Meng Zhang

Specialty type: Oncology

Provenance and peer review:

Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade B, Grade C

Novelty: Grade C, Grade C

Creativity or Innovation: Grade C, Grade D

Scientific Significance: Grade B, Grade C

P-Reviewer: Deng ZT, PhD, Associate Chief Physician, China

Received: October 9, 2025

Revised: November 5, 2025

Accepted: November 27, 2025

Published online: February 15, 2026

Processing time: 121 Days and 8.8 Hours



Ting-Ting Zhang, Han-Meng Zhang, Department of Oncology, Huai'an Hospital of Huai'an City, Huai'an 223299, Jiangsu Province, China

Juan Yao, Department of Radiation Oncology, Huai'an Hospital of Huai'an City, Huai'an 223299, Jiangsu Province, China

Co-corresponding authors: Juan Yao and Han-Meng Zhang.

Corresponding author: Han-Meng Zhang, MD, Department of Oncology, Huai'an Hospital of Huai'an City, No. 19 Shanyang Avenue, Huai'an 223299, Jiangsu Province, China. 15380666158@163.com

Abstract

This letter evaluates Deng *et al* study examining the gut microbiota and metabolite changes in metastatic colorectal cancer (CRC). The research used 16S rRNA sequencing and liquid chromatography-mass spectrometry metabolomics to investigate microbial and metabolic shifts in patients with metastatic *vs* non-metastatic CRC. The study reveals that CRC patients with metastasis exhibit significant differences in their gut microbiota and metabolites compared to non-metastatic patients. However, the study's reliance on 16S rRNA sequencing presents inherent limitations, particularly with respect to species-level resolution. The sequencing depth may not have been sufficient to capture all relevant low-abundance taxa, as indicated by the rarefaction curves which did not fully plateau, potentially affecting the identification of differential species. It also identifies 91 differential metabolites, particularly those involved in nucleic acid, alkaloid, and lipid metabolism, which may contribute to metastasis progression. The findings suggest that microbiota and their metabolites play a critical role in CRC metastasis, offering potential targets for diagnosis and treatment. However, several limitations exist, including small sample size, single-center data, and a cross-sectional design that prevents causal conclusions. Additionally, the study lacks integration of key clinical factors such as dietary patterns and medication use, which could confound the results. Future research should expand these findings through multi-center studies with longer follow-up periods, incorporating more comprehensive clinical data and advanced analytical techniques to validate and refine the role of microbiota and metabolites in CRC metastasis. Despite its limitations, this study provides valuable insights into the microbiota-metabolite axis in CRC metastasis and opens potential avenues for future research. However, it is crucial to note that the metabolite identification was based on database

matching rather than chemical standard validation. As such, these results should be considered putative annotations, with their accuracy requiring further confirmation through targeted analyses.

Key Words: Colorectal cancer; Gut microbiota; Metastasis; Metabolomics; 16S rRNA sequencing; Microbial diversity; Differential metabolites; Cancer progression; Metabolic pathways; Diagnostic biomarkers

©The Author(s) 2026. Published by Baishideng Publishing Group Inc. All rights reserved.

Core Tip: Deng *et al* explores the role of gut microbiota and metabolites in colorectal cancer (CRC) metastasis using 16S rRNA sequencing and liquid chromatography-mass spectrometry-based metabolomics. It identifies significant differences in microbial communities and metabolites between metastatic and non-metastatic CRC patients. The findings suggest that specific microbial taxa and metabolites may contribute to metastasis progression, offering potential biomarkers for diagnosis and treatment. Despite some limitations, such as small sample size and cross-sectional design, the study provides valuable insights into the microbiota-metabolite axis and its potential for therapeutic targeting in CRC.

Citation: Zhang TT, Yao J, Zhang HM. Microbiota-metabolite signatures in metastatic colorectal cancer: Promise, pitfalls, and the path forward. *World J Gastrointest Oncol* 2026; 18(2): 115010

URL: <https://www.wjgnet.com/1948-5204/full/v18/i2/115010.htm>

DOI: <https://dx.doi.org/10.4251/wjgo.v18.i2.115010>

TO THE EDITOR

The gut microbiota is increasingly recognized as a critical determinant in colorectal cancer (CRC) initiation, progression, and metastasis. Dysbiosis can perturb host metabolism, immune responses, and epithelial barrier integrity, thereby modulating tumor-host crosstalk. Integrative omics approaches – especially combining 16S rRNA sequencing with metabolomics – now afford deeper resolution of microbiota-metabolite networks in cancer biology. In this context, a recent study employed these methods to compare microbial and metabolic signatures between metastatic and non-metastatic CRC, identifying 91 differential metabolites and genus-level shifts suggestive of a microbiota-metabolite axis in metastasis. While provocative, the findings remain associative and warrant deeper methodological and translational scrutiny[1].

MICROBIOTA-METABOLITE SIGNATURES IN METASTATIC CRC: NEXT STEPS

This work showcases the promise of linking microbial taxa to specific metabolites, but several enhancements could sharpen inference and applicability. First, the single-center, limited-sample design restricts generalizability; replication across cohorts with standardized protocols is vital. Second, correlation networks should evolve into integrative multi-omics modeling (*e.g.*, sparse canonical correlation, DIABLO) to derive stable microbial-metabolite signatures predictive of metastatic phenotypes. Third, 16S rRNA sequencing offers only genus-level resolution; coupling with shotgun metagenomics or meta transcriptomics could map functional pathways directly tied to metabolites (*e.g.*, short-chain fatty acid production, bile acid transformation)[2,3]. Fourth, cross-sectional sampling hinders causal inference. Prospective sampling before metastasis occurrence or treatment response would clarify temporal dynamics, while animal or organoid models colonized with patient-derived microbiota could allow mechanistic testing[4]. Fifth, for clinical translation, the candidate genera (*e.g.*, *Butyrivibrio*, *Coprococcus*) and metabolites should be validated in external cohorts, with their discriminatory performance assessed (area under the curve, calibration, decision-curve analysis)[5,6]. Integrating omics signatures with standard clinical prognostic markers (*e.g.*, carcinoembryonic antigen, tumor stage) could yield composite risk models[7].

The study identified several differential genera and metabolites between metastatic and non-metastatic CRC groups. Notably, the study reported non-significant α -diversity but significant β -diversity, a common finding in microbial ecology. While species richness (α -diversity) remains similar between groups, the community structure (β -diversity) can still differ, emphasizing the need to focus on specific taxa rather than overall richness. Genera enriched in the non-metastatic group, such as *Butyrivibrio*, *Ruminococcus-1*, and *Coprococcus-2*, are well-known butyrate producers. The reduction of these genera suggests a potential loss of butyrate – a short-chain fatty acid with established anti-inflammatory, barrier-strengthening, and anti-cancer properties. These genera are not merely beneficial bacteria but represent functionally defined protective consortia that are linked to the prevention of CRC progression. In contrast, genera enriched in the metastatic group, including *Pyramidobacter*, *Christensenellaceae-R-7* group, and *Romboutsia*, are still ambiguous in their roles in CRC. These genera should be regarded as candidate pro-carcinogenic bacteria that require functional validation to confirm their potential involvement in CRC progression. Additionally, when analyzing a large number of operational taxonomic units and metabolites, the false discovery rate correction is essential to reduce the risk

of false positives. It is important to consider whether this correction was applied in the study's statistical analysis.

The study identified several metabolites, including diazoxide, hydroquinidine, aurapten, and triptophenolide, which are associated with CRC progression. However, these metabolites are not typical core metabolites of gut bacteria; they are more likely to be products of host metabolism, pharmaceutical derivatives, or dietary components modified by the microbiota[8]. This suggests a host-microbiota-xenobiotic interaction model that is more complex than previously considered. In addition, other well-known microbial metabolites, such as formate (derived from *Fusobacterium nucleatum*), secondary bile acids, hydrogen sulfide, and trimethylamine N-oxide, have been implicated in CRC tumorigenesis and metastatic progression through various mechanisms, including AhR signaling and glutamine metabolism. While some studies suggest anti-cancer effects of metabolites like diazoxide and hydroquinidine, the primary evidence comes from *in vitro* pharmacological models, and their actual physiological concentrations and biological effects within the human gut microenvironment, particularly in the context of CRC metastasis, remain uncertain. These findings highlight the need for direct evidence to better understand the role of microbial and microbial-modified metabolites in CRC progression, moving from associative findings to causative mechanisms *in vivo*[9,10]. The gut-microbiota-metabolite axis thus offers biologically plausible routes linking microbial dysbiosis to metastasis. Moreover, stage-specific microbiota and metabolite shifts reported across studies reinforce the validity of stratified analyses, though population heterogeneity, diet, and geography introduce variability that must be addressed through harmonized multi-cohort designs[11].

The study provides interesting findings, but the results were presented with limited reference to figures (original text), making it harder to interpret the significance of key results. To improve clarity, we reference specific figures (original text) such as the Venn diagram (Figure 1), principal component analysis plots (Figure 4), and heatmaps (Figures 6 and 7) when discussing microbial diversity and metabolic changes. These figures (original text) help contextualize the findings and enhance the persuasiveness of the results. The data visualization presented in the study was adequate but could be improved for better clarity. For instance, Figure 7 (original text) (the genus-level heatmap) employs a high-contrast dichromatic scheme that, while effective in highlighting broad inter-group differences, obscures the distribution patterns of genus abundances within each group. A continuous color gradient would more precisely display microbial abundance gradients, helping to assess within-group heterogeneity and reveal subtle relationships between samples and genera.

While the study presents intriguing results, several limitations, including the small sample size, cross-sectional design, and lack of metagenomic confirmation for the implicated microbial species' genetic capacity to produce or transform the identified metabolites (*e.g.*, diazoxide, hydroquinidine), should be considered in future research. The absence of causal confirmation through more direct methods, such as animal models or organoid systems, represents a critical gap between associative and mechanistic research. Additionally, the principal component analysis analysis revealed that the low variance contributions of PC1 and PC2 (approximately 10%) suggest that there is considerable unmodeled variation within the data, likely arising from individual differences, diet, and environmental factors, which weakens the confidence in these metabolites as robust biomarkers.

CONCLUSION

The study advances our understanding of the gut microbiota-metabolite interplay in CRC metastasis, highlighting new avenues for biomarker discovery and therapeutic intervention. To progress from association to action, future studies should integrate metagenomics to reveal microbial gene functions, meta transcriptomics to uncover actively expressed genes, and targeted metabolomics to precisely quantify key metabolites. These techniques, when combined, will provide a more comprehensive view of how microbial communities and their metabolites contribute to CRC metastasis. Furthermore, researchers should validate their findings using humanized mouse models by transplanting fecal microbiota from CRC patients into germ-free or antibiotic-treated mice and observing the resulting metastasis phenotypes. This direct validation can offer stronger evidence of the microbiota's functional role in metastasis. Lastly, future studies should collect detailed data on diet and medication and incorporate these as covariates in statistical models. This will help disentangle the independent contributions of microbial and metabolite changes, providing more precise insights into their role in CRC progression. Ultimately, defining microbial metabolite drivers of metastasis could open novel microbiome-guided strategies for prognosis, prevention, and treatment of metastatic CRC.

FOOTNOTES

Author contributions: Zhang TT was responsible for conceptualization, methodology, data curation, and writing original draft; Yao J and Zhang HM were responsible for conceptualization, investigation, writing review and editing, supervision, methodology, validation, and supervision as co-corresponding authors; all of the authors read and approved the final version of the manuscript to be published.

Conflict-of-interest statement: All authors declare no conflict of interest in publishing the manuscript.

Open Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <https://creativecommons.org/licenses/by-nc/4.0/>

Country of origin: China

ORCID number: Juan Yao 0000-0001-8515-9106; Han-Meng Zhang 0009-0004-6692-0341.

S-Editor: Luo ML

L-Editor: A

P-Editor: Zhao S

REFERENCES

- 1 **Deng P**, Lin H, Zeng H, Guo F. Gut microbiota and metabolite changes in metastatic colorectal cancer *via* 16S rRNA and metabolomics. *World J Gastrointest Oncol* 2025; **17**: 111509 [RCA] [PMID: 41114121 DOI: 10.4251/wjgo.v17.i10.111509] [FullText] [Full Text(PDF)]
- 2 **Chen W**, Zhang P, Zhang X, Xiao T, Zeng J, Guo K, Qiu H, Cheng G, Wang Z, Zhou W, Zeng S, Wang M. Machine learning-causal inference based on multi-omics data reveals the association of altered gut bacteria and bile acid metabolism with neonatal jaundice. *Gut Microbes* 2024; **16**: 2388805 [RCA] [PMID: 39166704 DOI: 10.1080/19490976.2024.2388805] [FullText] [Full Text(PDF)]
- 3 **Yachida S**, Mizutani S, Shiroma H, Shiba S, Nakajima T, Sakamoto T, Watanabe H, Masuda K, Nishimoto Y, Kubo M, Hosoda F, Rokutan H, Matsumoto M, Takamaru H, Yamada M, Matsuda T, Iwasaki M, Yamaji T, Yachida T, Soga T, Kurokawa K, Toyoda A, Ogura Y, Hayashi T, Hatakeyama M, Nakagama H, Saito Y, Fukuda S, Shibata T, Yamada T. Metagenomic and metabolomic analyses reveal distinct stage-specific phenotypes of the gut microbiota in colorectal cancer. *Nat Med* 2019; **25**: 968-976 [RCA] [PMID: 31171880 DOI: 10.1038/s41591-019-0458-7] [FullText]
- 4 **Liu JL**, Xu X, Rixiati Y, Wang CY, Ni HL, Chen WS, Gong HM, Zhang ZL, Li S, Shen T, Li JM. Dysfunctional circadian clock accelerates cancer metastasis by intestinal microbiota triggering accumulation of myeloid-derived suppressor cells. *Cell Metab* 2024; **36**: 1320-1334.e9 [RCA] [PMID: 38838643 DOI: 10.1016/j.cmet.2024.04.019] [FullText]
- 5 **Sun L**, Zhu Z, Jia X, Ying X, Wang B, Wang P, Zhang S, Yu J. The difference of human gut microbiome in colorectal cancer with and without metastases. *Front Oncol* 2022; **12**: 982744 [RCA] [PMID: 36387258 DOI: 10.3389/fonc.2022.982744] [FullText]
- 6 **Gao R**, Wu C, Zhu Y, Kong C, Zhu Y, Gao Y, Zhang X, Yang R, Zhong H, Xiong X, Chen C, Xu Q, Qin H. Integrated Analysis of Colorectal Cancer Reveals Cross-Cohort Gut Microbial Signatures and Associated Serum Metabolites. *Gastroenterology* 2022; **163**: 1024-1037.e9 [RCA] [PMID: 35788345 DOI: 10.1053/j.gastro.2022.06.069] [FullText]
- 7 **Li X**, Ramadori P, Pfister D, Seehawer M, Zender L, Heikenwalder M. The immunological and metabolic landscape in primary and metastatic liver cancer. *Nat Rev Cancer* 2021; **21**: 541-557 [RCA] [PMID: 34326518 DOI: 10.1038/s41568-021-00383-9] [FullText]
- 8 **Ternes D**, Tsenkova M, Pozdeev VI, Meyers M, Koncina E, Atatri S, Schmitz M, Karta J, Schmoetten M, Heinken A, Rodriguez F, Delbrouck C, Gaigneaux A, Ginolhac A, Nguyen TTD, Grandmougin L, Frachet-Bour A, Martin-Gallausiaux C, Pacheco M, Neuberger-Castillo L, Miranda P, Zuegel N, Ferrand JY, Gantenbein M, Sauter T, Slade DJ, Thiele I, Meiser J, Haan S, Wilmes P, Letellier E. The gut microbial metabolite formate exacerbates colorectal cancer progression. *Nat Metab* 2022; **4**: 458-475 [RCA] [PMID: 35437333 DOI: 10.1038/s42255-022-00558-0] [FullText] [Full Text(PDF)]
- 9 **Pérez Escriba P**, Correia Tavares Bernardino C, Letellier E. De-coding the complex role of microbial metabolites in cancer. *Cell Rep* 2025; **44**: 115358 [RCA] [PMID: 40023841 DOI: 10.1016/j.celrep.2025.115358] [FullText]
- 10 **Yang YC**, Chang SC, Hung CS, Shen MH, Lai CL, Huang CJ. Gut-Microbiota-Derived Metabolites and Probiotic Strategies in Colorectal Cancer: Implications for Disease Modulation and Precision Therapy. *Nutrients* 2025; **17**: 2501 [RCA] [PMID: 40806086 DOI: 10.3390/nu17152501] [FullText] [Full Text(PDF)]
- 11 **Fan S**, Zhou L, Zhang W, Wang D, Tang D. Role of imbalanced gut microbiota in promoting CRC metastasis: from theory to clinical application. *Cell Commun Signal* 2024; **22**: 232 [RCA] [PMID: 38637851 DOI: 10.1186/s12964-024-01615-9] [FullText] [Full Text(PDF)]



Published by **Baishideng Publishing Group Inc**
7041 Koll Center Parkway, Suite 160, Pleasanton, CA 94566, USA

Telephone: +1-925-3991568

E-mail: office@baishideng.com

Help Desk: <https://www.f6publishing.com/helpdesk>

<https://www.wjgnet.com>

