



Revisiting multi-region 16S sequencing in gastric cancer

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Abstract

Wu *et al* recently applied multi-region 16S rRNA sequencing to characterize the gastric cancer microbiome, demonstrating improved taxonomic resolution and detection sensitivity over conventional single-region approaches. While the study represents a valuable methodological step forward, it remains limited by single-center design, lack of quantitative calibration, and insufficient control for contamination and inter-laboratory variability. This editorial critically appraises these methodological gaps and emphasizes that future efforts must focus on harmonized, consensus-driven workflows to ensure reproducibility and clinical reliability. The translational potential of multi-region 16S lies in moving from descriptive microbial profiling to actionable clinical integration, particularly for recurrence prediction, treatment-response monitoring, and perioperative complication risk assessment. By addressing these methodological, economic, and ethical challenges, the field can advance toward evidence-based and clinically deployable microbiome-guided precision oncology.

Key Words: Gastric cancer; Microbiome; Multi-region 16S rRNA sequencing; Metagenomics; Biomarkers; Prognosis; Immune microenvironment; Precision oncology

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Core Tip: Multi-region 16S rRNA sequencing represents a promising advance in gastric cancer microbiome research, improving microbial detection and taxonomic resolution compared to traditional methods. However, its clinical utility hinges on rigorous validation, integration with metagenomics and host factors, and the inclusion of health economic analyses. Future studies should aim to transform this technology from descriptive microbial profiling into a decision-support tool for prognosis, perioperative risk management, and personalized therapeutic strategies, overcoming existing methodological and translational challenges.

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INTRODUCTION

In recent years, tumor-associated microbiota research has emerged as an important frontier at the intersection of oncology and microbiome science[1,2]. This is particularly relevant in gastric cancer (GC), a malignancy of the digestive system characterized by strong heterogeneity and insidious early symptoms, where the search for novel, reproducible, and translatable biomarkers is especially urgent[3]. Gastric-colonizing bacteria have been shown to influence tumor initiation, progression, and therapeutic responses through mechanisms involving metabolites, inflammatory regulation, and immune reprogramming[4]. Traditional single-region 16S rRNA sequencing faces limitations in detecting low-abundance taxa and resolving complex microbial communities; therefore, the development of higher-resolution and broader-coverage sequencing strategies is of great importance[5].

Recently, Wu *et al*[6] reported in the *World Journal of Gastrointestinal Oncology* the application of multi-region 16S rRNA gene sequencing in the analysis of GC microbiota. Their findings demonstrated that, compared with conventional single-region sequencing, sequencing across multiple hypervariable regions significantly improves sensitivity and taxonomic resolution. This advance is noteworthy in the context of tumor-associated microbiota research, particularly for low-biomass and formalin-fixed paraffin-embedded (FFPE)-preserved tissue samples that are frequently encountered in clinical practice. The authors highlighted the utility of this approach in improving microbial diversity profiling, thus providing a refined ecological landscape and laying a methodological foundation for further exploration of microbiota-related carcinogenesis. However, translating such microbiome profiling into clinically reliable tools remains an unresolved challenge, particularly in linking microbial diversity with actionable outcomes.

ANALYZE AND CRITICALLY EVALUATE

The study was conducted in a single center with a retrospective design, limiting the generalizability of its conclusions. Multi-center, prospective cohorts with more diverse populations are needed to validate these findings[7].

Although increased species richness and diversity were reported, the absence of quantitative calibration using qPCR or spike-in standards raises concerns about low-abundance taxa. Incorporating absolute quantification strategies would improve reliability[8].

Contamination control and batch-effect handling were insufficiently described, which is critical for low-biomass samples such as FFPE tissues. The systematic use of negative controls and appropriate decontamination pipelines (*e.g.*, Decontam, KneadData) could further enhance data reliability and reproducibility[9,10]. Moreover, inter-laboratory variability and the absence of standardized sequencing protocols remain potential sources of inconsistency, underscoring the need for harmonized workflows to ensure reproducibility across studies[11].

Finally, analyses relied solely on relative abundance, which may introduce compositional bias. Future studies should integrate absolute abundance measures and functional prediction tools to provide more accurate ecological and mechanistic insights[12,13].

FROM “MEASURABLE” TO “ACTIONABLE”: REASSESSING TRANSLATIONAL VALUE

The core contribution of this study lies in extending the methodological boundaries of GC microbiome research. However, its broader clinical potential has yet to be fully realized. We believe that the value of multi-region 16S rRNA sequencing should not remain confined to descriptive microbial diversity, but rather be directed toward patient-centered translational applications.

Clinical risk prediction

Fusobacterium nucleatum and other taxa have been linked to recurrence, immune evasion, and chemoresistance in GC[14, 15]. Integrating microbial signatures with host and clinical factors may enable composite prognostic models validated by

Cox regression or decision curve analysis.

Perioperative risk monitoring

Microbial imbalance is associated with postoperative complications such as infection and anastomotic leakage. Combining preoperative microbiota profiles with inflammatory markers (*e.g.*, C-reactive protein, neutrophil-to-lymphocyte ratio) could support early warning and perioperative risk assessment[16].

Subtype and metabolic profiling

Distinct microbial patterns across Lauren types and TNM stages have been reported[17-19]. Integrating multi-region 16S with shallow metagenomics could reveal metabolic pathways underlying histological subtypes and inform precision therapy[19,20]. In this way, the true value of multi-region 16S lies in its integration with metagenomics and host factors, enabling a transition from being merely “measurable” to becoming truly “actionable” in risk prediction, complication management, and subtype-specific research.

FUTURE RESEARCH DIRECTIONS

Future studies should first be strengthened at the design level. Large-scale validation cohorts, combined with subgroup analyses that incorporate host immune characteristics, will be essential to enhance robustness and generalizability[21].

On this foundation, research should integrate multi-region 16S with shallow metagenomics[22]. Such integration can overcome the functional limits of a single approach and clarify the contributions of metabolism, drug resistance, and signaling pathways in gastric carcinogenesis[20]. Evidence indicates that reproducible microbial patterns exist across pathological stages, supporting the feasibility of incorporating microbial features with clinical staging and pathology into risk stratification models[21]. Application of methods such as LASSO, XGBoost, and decision curve analysis not only enhance prediction accuracy but also facilitate translational model deployment by identifying clinically meaningful microbial signatures.

Once predictive models are validated, attention should turn to interventional strategies. Dietary modification or probiotic supplementation has been suggested to reshape gastric microbiota and attenuate inflammation, indicating that future trials combining such interventions with dynamic monitoring *via* multi-region 16S may provide empirical evidence of microbial plasticity and its preventive and therapeutic potential[23,24].

Finally, the pathway to clinical translation will require health economic evaluations, defined as assessments comparing clinical outcomes and financial costs, to assess cost and clinical benefit trade-offs. Comparing the cost-effectiveness of multi-region 16S with single-region sequencing, metagenomic approaches, and conventional biomarkers across different clinical settings will be critical to define its real-world value and to support evidence-based precision oncology[25].

CONCLUSION

The study by Wu *et al*[6] highlights the technical advantages and potential of multi-region 16S rRNA sequencing in GC microbiome research. However, to move this approach from descriptive studies to a decision-making tool, more rigorous study designs and cross-disciplinary integration will be needed[26]. Future work should focus on multicenter collaboration and standardized, consensus-driven multi-region 16S workflows. Harmonizing key elements like sample collection, DNA extraction, sequencing, and data analysis will ensure consistency and enhance clinical reliability across laboratories. Among potential clinical endpoints, recurrence prediction, treatment-response monitoring, and postoperative complication risk represent the most feasible for early clinical integration[27-29]. In addition, regulatory and ethical considerations, including data privacy, informed consent, and oversight of sequencing technologies, should be clarified to ensure biosafety and equitable access[30]. Only through such efforts can GC microbiome research evolve from a scientific hotspot into a deployable biomarker system, enabling precision oncology, improving patient stratification, and ultimately enhancing clinical outcomes[31].

FOOTNOTES

Author contributions: Luo L conceived the study design, drafted the initial manuscript, and revised the content critically; Huang G contributed to literature review, data interpretation, and manuscript editing; Yang H contributed to data interpretation, discussion of methodological aspects, and manuscript refinement; Chi H supervised the overall concept, provided methodological guidance, and critically revised the manuscript as corresponding author; all authors have read and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

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