

The Application of Gut Microbiota in the Diagnosis, Prognosis and Treatment of Colorectal Cancer

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Abstract. The gut microbiota plays a significant role in the occurrence and development of colorectal cancer (CRC), and its application value in the diagnosis and treatment of CRC has received extensive attention in recent years. The gut microbiota has shown great potential as a non-invasive biomarker and therapeutic target in CRC. However, existing reviews mostly focus on mechanism descriptions and lack systematic analysis of clinical translation barriers. This article focuses on CRC and systematically reviews the research progress of the microbiota as a diagnostic biomarker, prognostic biomarker, and therapeutic target, and emphasizes the discussion of translation barriers and solutions. In terms of diagnosis, fecal microbiota testing faces the lack of standardization and interference from confounding factors. About prognosis, the unclear causal relationship and insufficient standardization of bioinformatics limit clinical application. In terms of treatment, fecal microbiota transplantation and probiotics and other intervention strategies are limited by individual variability, regulatory barriers, and insufficient strain specificity. This article proposes three major solution paths: standardization construction, multi-omics integration, and precise intervention, providing a reference for the clinical translation of microbiota research results.

Keywords: Gut microbiota; colorectal cancer; biomarker; fecal microbiota transplantation.

1. Introduction

Colorectal cancer (CRC) ranks in third place in terms of incidence but second in terms of mortality, with the incidence rate on the rise [1]. Although the widespread implementation of these examinations, like direct visualization tests (e.g. colonoscopy, flexible sigmoidoscopy, colonography) or stool-based tests (e.g. guaiac fecal occult blood test [gFOBT], fecal immunochemical test [FIT]), or blood-based tests (e.g. methylated SEPT9 gene) tests, has to some extent reduced the mortality rate, the adherence with screening has always lagged behind. There are also problems such as high resource consumption, high risks, or low detection rates [2].

In recent years, with the development, popularization and progress, a large number of studies have confirmed the gut microbiota plays an essential role in the initiation and progression of CRC, which shows the enrichment of species such as *Fusobacterium nucleatum* subsp. *animalis* clade 2 (Fna C2) [3], and shows great potential as a non-invasive biomarker in early screening, prognosis assessment and treatment response to be a supplementary screening tool. Studies have shown that gut microbiota and its metabolites can alter gene expression, signaling pathways, leading to gut dysbiosis, changing the microenvironment, and promoting the occurrence of CRC [3,4]. After surgical resection, gut dysbiosis persists and affects the prognosis and survival of CRC patients [5-7]. These changes can be captured and applied to early screening and prognosis assessment, providing treatment strategies for CRC patients as early as possible.

At the same time, the gut microbiota intervention strategies, including fecal microbiota transplantation (FMT) and probiotics and their metabolites are also moving from basic research to clinical practice [8-10], providing precise and personalized treatment for CRC patients. However, most of the existing reviews mainly focus on the description of mechanisms and lack in-depth analysis of the key obstacles in the clinical translation process. Based on this, this article systematically reviews the research progress of the gut microbiota as diagnostic markers, prognostic markers, and therapeutic targets, and also incorporates the challenges and solutions for the translation from the laboratory to clinical practice.

2. Gut Microbiota as a Diagnostic Biomarker

2.1. Fecal Microbiota Testing for Early CRC Screening

The traditional screening methods of CRC mainly include colonoscopy and FIT. Although colonoscopy is the gold standard, it has limitations such as invasiveness, high cost, and poor patient adherence. And detection sensitivity for advanced adenomas of FIT is relatively low (about 0.23-0.25) [2]. This means that it is mainly good at detecting existing cancers but is not good at capturing early precancerous lesions.

Fecal microbiota testing as a non-invasive examination has great potential to be a supplementary screening tool to avoid unnecessary colonoscopy and also enhance the detection rate of cancer. The Fna C2 branch of *Fusobacterium nucleatum* can lead to glutathione (GSH) depletion and alter the local metabolic microenvironment. This metabolic disorder influences the entire microbiota ecosystem and indirectly alters the bacterial community structure [3]. Further studies have confirmed that this microenvironmental change is accompanied by significant enhancement of metabolic pathways. Given this "microbiota-metabolite-microenvironment" chain, detecting these specific metabolic functional features, based on functional gene markers, can more accurately capture early pathological changes. As the research found, there were extremely strong diagnostic capabilities in the detection of CRC (area under the receiver operating characteristic curve (AUROC) = 0.86). At the same time, this study also achieves great diagnostic value about specific microbiota abundance (AUROC=0.84), and early diagnosis (AUROC=0.96) [4], which indicates a considerable diagnostic possibility.

2.2. Value of Microbiota Features in Adenoma-Carcinoma Differentiation

Early identification of adenoma is the key to preventing and treating CRC. Research found the gut microbiota undergoes staged changes during the progression of CRC. The phylum *Fusobacteria* is significantly enriched only during the cancer stage, not during the adenoma stage. Adenomas have unique microbial characteristics: compared to healthy controls, the beneficial bacteria such as *Bifidobacterium longum* are depleted; compared to cancer, the abundance of pathogenic bacteria such as *Escherichia-Shigella* sp. is significantly different. And the research has 11 markers to discriminate adenoma from control (area under the ROC curve (AUC) = 0.80), and 26 markers to discriminate adenoma from CRC (AUC = 0.89), respectively [11].

And it was also confirmed that gut microbiota not only demonstrated excellent diagnostic capabilities when compared with healthy controls and CRC, with an AUC of 0.90 and an extremely high specificity (0.97), but also it performed strongly in differentiating adenomas from CRC, with an AUC of 0.90. However, its ability to capture early adenoma lesions is limited (AUC 0.63) [12]. This difference may show that the ability of gut microbiota to distinguish between adenomas and health control has heterogeneity. Meanwhile it also reflects the dysbiosis at the adenoma stage is still in its early stage and the changes are relatively mild. Although gut microbiota detection has value in adenoma-carcinoma differentiation, the adenoma identification ability still needs to be improved. And that means gut microbiota detection could only be diagnostic assistance tool instead of replacing colonoscopy.

2.3. Clinical Translation Barriers and Solutions for Microbiota-Based Diagnostic Biomarker

2.3.1. Main Clinical Translation Barriers

Though gut microbiota as the diagnostic biomarker has shown great potential, it still faces various barriers. Including the absence of standardization, variability between individuals and costs problem. In terms of lack of standardization, at the technical and analytical levels, different studies have varying experimental and analytical methods, which can lead to different microbiota profiles and biases [4,12]. There is heterogeneity in the composition and function of the microbiota. The gut microbiome is strongly influenced by dietary habits, living environments, and the host's genetic background, resulting in significant differences in microbiota structure among different populations,

making it difficult to establish universal diagnostic criteria [4,11]. In terms of economic and implementation aspects, for early screening to be carried out on a large scale among the population, the issue of cost needs to be taken into account.

2.3.2. Suggestions for the Clinical Barriers of Microbiota-Based Diagnostic Biomarker

In order to solve the technical biases, the standardized bioinformatics analysis process and calibration algorithm can be used [12]. For overcoming the heterogeneity of the population, the leaveable dataset (LODO) or the integrated scoring system (such as a microbial risk score) is built to integrate the multi-kingdom features of bacteria, fungi, and viruses [4,11,12]. At the implementation level, the combination of microbial testing with existing fit screening is a feasible strategy for consideration of sensitivity and cost effectiveness [11,12]

3. Gut Microbiota as a Prognostic Biomarker

3.1. Association Between Microbiota Features and Postoperative Recurrence

Postoperative recurrence in CRC patients is a key factor influencing prognosis. Regarding the underlying mechanism, CRC patients exhibited significant dysbiosis before surgery, with increased abundance of potential pathogenic bacteria such as *Bacteroides* species. Although beneficial bacteria like *Bifidobacterium* recovered post-surgery, it still lasts for 6 months after the operation that the characteristics of cancer-related dysbiosis persisted. This persistent dysbiosis may be related to the recurrence risk of CRC [5].

In terms of postoperative monitoring, the study shows that although the tumor was removed after the surgery, it would lead to a further decline in the diversity of the microbiota. More importantly, the follow-up data indicate that the abnormal rebound of *Fusobacterium* and the continuous absence of beneficial bacteria are closely related to the recurrence or death risk of the patients. Therefore, fecal microbiota testing is also an important non-invasive tool for monitoring postoperative prognosis and warning of recurrence [6].

In terms of preoperative prediction, the research found that *Prevotella* has a high abundance and is significantly associated with a lower recurrence risk and better survival rate. While the presence of *Fusobacterium nucleatum* and three newly identified pathogens indicates a high recurrence risk. The microbial risk score (M5) constructed based on these five bacteria can precisely classify patients into different risk levels, and its ability to predict recurrence is superior to traditional clinical indicators. And that proved preoperative bacterial community status can serve as a non-invasive tool to guide personalized management and intervention after surgery [13].

3.2. Association Between Microbiota and Patient Survival

There is a complex relationship between the composition of gut microbiota and the survival of CRC patients, and its specific relationship depends on the specific type of gut microbiota, the microenvironment of the group and the clinical indicators of the evaluation.

There is a clear negative survival predictor, such as *Streptomyces lividans* and the *pseudomonas* sp., is significantly associated with the reduction of overall survival (OS), which tends to reduce the survival of the patient by building a inflammatory environment or promoting tumor progress [7]. And the increased number of *Prevotella*, which increases with age, is also confirmed to increase the risk of death [14]. That is because *Prevotella intermedia* and *Fusobacterium nucleatum* work together to drive adenomas into cancer [15]. The research has found that the nuclear membrane bacteria, which are usually regarded as pathogenic, may strongly activate the immune system due to the formation of immune memory caused by the body's immune response. This effectively inhibits the recurrence of tumors. [16] Therefore, the gut bacteria can not only affect the OS by direct cancer or cancer suppression but also determine survival period by regulating the host immune state, which emphasizes the important value of its prognosis in cancer.

3.3. Clinical Translational Barriers and Solutions for Microbiota-Based Prognostic Biomarker

3.3.1. Main Clinical Translational Barriers

There are many obstacles in the clinical translation of gut microbiota as prognostic markers for CRC. Firstly, the lack of standardization in sample collection and processing, combined with confounding factors like diet and antibiotics, severely limits data comparability [5,6,16]. Secondly, the inherent complexity and dynamic nature of the microbial community (which is exacerbated by surgery, age, and significant individual differences) increases the difficulty of detection [5,6,13]. Thirdly, the current research mainly relies on 16S rRNA sequencing, lacking validation through metagenomics or metabolomics to establish an accurate causal relationship mechanism [5,7]. Finally, there is insufficient clinical integration and model validation. Existing prognostic models are often overly complex and costly, lacking large-scale, multi-center prospective validation [5-7]. Therefore, based on these studies, gut microbiota as the prognostic biomarker is difficult to implement in clinical practice and widely used in hospitals.

3.3.2. Solution for the Translational Barriers of Microbiota-Based Prognostic Biomarker

Facing the current obstacles in clinical translation, the research methods will also undergo changes. To address the issue of existing models being overly complex and expensive, it can use machine learning algorithms to screen large amounts of data and select the most significant few markers. It can construct a more cost-effective and efficient screening model, which greatly enhances the applicability of the technology in the routine clinical environment [7]. Regarding the inconsistency in the prognostic of markers among different studies, treatment can be tailored based on classification. By identifying specific bacterial combinations, patients can be classified, thereby recognizing the role that the same bacteria may play in different patient groups. This analysis method based on specific bacterial combination explains the heterogeneity of markers and improves the accuracy of prognosis judgment [13,16]. For the lack of large-scale validation, more rigorous research methods can be adopted, such as transitioning from retrospective studies to large-scale, multi-center prospective clinical trials. This will better ensure the validity of the model. In terms of the insufficient clinical integration and validation, by establishing global unified sample collection and data analysis norms, it can effectively reduce differences between different studies, ensuring the reproducibility and generalizability of the model.

4. Gut Microbiota as a Therapeutic Target

4.1. Modulating Gut Microbiota to Enhance Chemotherapy or Immunotherapy Efficacy

The core mechanism of enhancing the efficacy of chemotherapy and immunotherapy by regulating the gut microbiota lies in reshaping the tumor microenvironment (TME) and modulating host metabolism. Research has found that specific probiotic communities or their metabolic products can directly activate anti-tumor immunity. In mouse experiments, the combination of Prevotellaceae and anti-PD-L1 antibodies demonstrated the strong tumor suppression effect [17].

A Tumor-Suppressing Multi-Enterobacteria (TSME) composed of nine probiotics reshaped the gut microbioma in mouse models, enriching beneficial bacteria, transforming the cold TME into a hot tumor. It also significantly increased CD8⁺ T cell infiltration and upregulated immune pathways to enhance the efficacy of anti-PD-1/PD-L1 in microsatellite stable (MSS) CRC [8].

Meanwhile, specific bacteria within tumors can also sensitize chemotherapy combined with immunotherapy through epigenetic mechanisms. Research in mouse models found that enterotoxigenic *Bacteroides fragilis* (ETBF) was enriched when capecitabine was combined with PD-1 inhibitors, thereby promoting the expansion of CD8⁺ T cells and reversing the exhaustion of T cells. And ultimately, it significantly increased treatment sensitivity. Subsequently, in clinical trials, this

was validated in a cohort of CRC patients, confirming a strong correlation between SphK2 activity and patient survival benefits [18].

4.2. Application of Fecal Microbiota Transplantation in CRC Adjuvant Therapy

FMT as a method to restore intestinal microecology, mainly by cooperating with immune checkpoint inhibitors (ICIs) to overcome drug resistance and improve prognosis, has shown significant potential in the adjuvant treatment of CRC.

FMT can significantly enhance the efficacy of anti-PD-1 therapy. In preclinical studies, using mouse models, the combination of FMT and anti-PD-1 can significantly inhibit the growth of colorectal tumors and prolong survival. The survival rate of mice in the combined treatment group reached 70%, far higher than 30% in the group treated with anti-PD-1 therapy alone [9]. And clinical evidence further supports this finding. A phase II trial targeting MSS metastatic CRC showed that FMT combined with anti-PD-1 and anti-angiogenic drug achieved a progression-free survival (PFS) of 29.6 months and OS of 52.8 months. The abundance of Lachnospiraceae in the feces of responders significantly increased, suggesting it could serve as a predictive marker of efficacy [10]. Meanwhile, a meta-analysis covering multiple studies also pointed out that FMT as an adjunctive approach in combination with ICIs achieved an objective response rate (ORR) of 20% [19].

4.3. Clinical Translational Barriers and Solutions for Microbiota-Based Interventions

4.3.1. Main Clinical Translational Barriers

Although gut microbiota interventions (such as FMT and probiotics) have shown great potential in enhancing the efficacy of ICIs, their clinical translation still faces many obstacles. There is insufficient clinical evidence. The results obtained from small-scale retrospective studies vary greatly [10,18]. The intervention methods also lack standardization. FMT has issues such as donor screening and pathogen risks [10,19]. While the optimal colonization, dosage and proportion of probiotics remain unclear. [8-10].

And the underlying mechanism of gut microbiota work is complex [18], with key genera exhibiting dual effects [9,17] and a lack of reliable biomarkers. There are host individual differences and environmental factors that interfere. The baseline microbiota structure of the host, dietary habits, antibiotic use history, and tumor type significantly affects the intervention effect [9,10,19], further increasing the difficulty of clinical application.

4.3.2. Suggestions for the Translational Barriers of Microbiota-Based Interventions

About the heterogeneity, it can carry out large-scale, multi-center randomized controlled trials (RCT) and build a unified data sharing platform to improve the quality of clinical evidence and reduce the heterogeneity of research [10,18]. For standardization, it can establish an internationally recognized standardized operation specification, covering the control of the supply and capsule's preparation and quality, and the safety monitoring system and the ethical review to ensure the safety of the measures such as FMT [10,19]. In mechanism research, it can further study the molecular mechanisms of the group-host interaction (such as the metabolic product slp and the epigenetic regulation [18], the remodeling of the immune microenvironment [17]), and the integrated multi-omics analysis to filter the high specificity of the prediction biomarker [9,19]. In addition, the development of the new probiotics or synthetic bacteria group (defined consortia) can help solve the standardization problem [8]. In clinical practice, the clinical practice of patients should be taken into consideration of the patient's baseline microbiota characteristics, the use of antibiotics and the background of the diet, and the individualized gut microbiota intervention scheme to maximize objective response rate (ORR) of immunotherapy [8-10].

5. Discussion

This review proposes the "gut microbiota metabolism-TME immunity-clinical outcome" framework. Within this framework, the gut microbiota influences the abundance and microenvironment through its metabolites, regulates the TME, and encompasses the diagnosis, prognosis, and treatment of CRC. The metabolites of the microbiota are the key link between the gut microbiota and TME. The altered microenvironment caused by the presence of *Fusobacterium* leads to dysbacteriosis, which in turn reshapes the TME. The short-chain fatty acids produced by them mainly inhibit histone deacetylases (HDAC) within CRC cells, induce DNA damage in cancer cells, and activate the cGAS/STING pathway. This process significantly upregulates the expression of MHC I molecules on tumor cells, thereby enhancing their ability to activate CD8⁺ T cells and triggering a strong anti-tumor immune response [20]. In terms of diagnosis, the establishment of TME immunity remodeling demonstrates excellent CRC diagnostic capabilities. In terms of prognosis, high abundance of nucleated rods has a high risk of postoperative recurrence. In terms of treatment, reshaping the intestinal microbiota through FMT or probiotic intervention to improve the sensitivity to immunotherapy.

Within this framework, the barriers to transformation are largely similar. The absence of standardized experiments, heterogeneity, and a lack of more studies (such as multi-omics studies, cross-cohort studies). In the future, more attention should be focused on causal relationship research between metabolites and TME, and more multi-omics and cross-cohort studies should be conducted. In terms of treatment, achieve precise intervention and the transformation from mouse models to clinical experiments.

6. Conclusion

This review systematically summarizes the evidence of the application of gut microbiota in the diagnosis, prognosis and treatment of CRC. It concludes that gut microbiota biomarkers perform exceptionally well in differentiating CRC from healthy controls (with an AUC>0.8), and fecal microbiota transplantation and other intervention strategies have shown preliminary efficacy in refractory patients (with an ORR of 20%), indicating a broad clinical application prospect.

However, based on the available evidence, this review concludes that this field is still in the early stage of clinical translation and does not yet meet the conditions for entering routine clinical practice. The reasons are as follows: First, technical limitations such as poor standardization and reproducibility. Second, inadequate clinical evidence due to a lack of large-scale prospective RCTs. Third, high upfront costs associated with establishing standardized cohorts and securing regulatory approval.

Therefore, the most appropriate positioning of microbiota detection at the current stage is as research and a clinical auxiliary tool, rather than an independent diagnostic method. This review believes that with the improvement of multi-center prospective cohorts, the standardization of metabolite detection, and the completion of more RCTs, gut microbiota detection is expected to truly enter clinical practice for CRC in the future, becoming an important auxiliary tool for screening, prognosis judgment, and individualized treatment.

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