Research advances in the role of circulating microorganisms in gastrointestinal tumors (Review)

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Abstract. Gastrointestinal tumors are common malignant tumors of the digestive system, which globally threaten human health. Notably, it has been discovered that blood and other circulating body fluids are not completely sterile; instead, they harbor complex and dynamic microbial DNA and signatures [circulating microorganisms (CM)]. These microorganisms primarily originate from the microbial translocation (including bacterial fragments, DNA and metabolites) through a compromised intestinal barrier, and are closely associated with the initiation and progression of gastrointestinal tumors, thus providing novel perspectives for early tumor diagnosis and prognosis. Although there is currently no evidence that CM can directly cause cancer, their metabolites and exosomes may contribute to tumor microenvironment remodeling. On one hand, they activate pattern recognition and inflammatory signaling pathways, such as Toll-like receptor/signal transducer and activator of transcription, potentially inducing and maintaining low-grade chronic inflammation. On the other hand, they may facilitate immune evasion, potentially promoting the 'inflammation-cancer' transition. With the development of metagenomic technologies and the maturation of next-generation high-throughput sequencing technologies, CM have shown potential as liquid biopsy biomarkers for the early diagnosis of gastrointestinal tumors. Interventions targeting specific CMs have also shown prospects for enhancing efficacy in early clinical trials. However, the field still faces numerous challenges, including insufficient depth of mechanistic validation and a lack of standardized detection protocols. Future efforts should aim to conduct further systematic research to clarify the biological functions and clinical translational value of CM in gastrointestinal tumors.

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Contents

1. Introduction

The latest statistics from the Global Burden of Cancer Data reveal that malignant tumors of the digestive system account for 26% of all new cancer cases yet cause 35% of cancer-related deaths, exhibiting the prominent characteristics of 'high incidence and even higher mortality' (1). Among these, gastric cancer, colorectal cancer, esophageal cancer, liver cancer, and pancreatic cancer are the primary pathological types. Ranked by mortality, colorectal cancer, liver cancer, and gastric cancer occupy the top three positions, accounting for 9.3, 7.8, and 6.8% of global cancer deaths, respectively (2). Tumor progression is a multistage process involving the gradual accumulation of genetic mutations and epigenetic alterations, with the tumor microenvironment playing a decisive role. Recent advances in mechanistic research reveal that the circulating microorganisms (CM) are associated with alterations in the tumor microenvironment by regulating inflammatory signaling and immune evasion pathways, potentially serving as a critical factor in the development and progression of gastrointestinal malignancies (3). Therefore, systematically deciphering the dynamic characteristics and functional mechanisms of CM in gastrointestinal malignancies not only deepens our understanding

of their pathogenesis but also provides potential targets for developing microbiome-targeted intervention strategies. In light of this, this review summarizes the latest research advances on the relationship between CM and the occurrence, progression, and outcomes of gastrointestinal tumors, aiming to provide evidence-based guidance and directional references for subsequent basic and clinical translational research.

In the context of this review, the term 'circulating microorganisms (CM)' refers broadly to microbial signatures detectable in blood and body fluids, including circulating microbial DNA (cmDNA), cell-free microbial RNA, circulating pathogen-associated molecular patterns (PAMPs), microbial metabolites (e.g., short-chain fatty acids, bile acids), and microbial extracellular vesicles (mEVs). It is critical to distinguish these molecular components from viable, culturable microbial cells. While translocation of intact bacteria into circulation may occur under pathological conditions (e.g., severe leaky gut), current metagenomic evidence predominantly detects microbial nucleic acids and products rather than living, replicating microbial communities in the bloodstream. Therefore, CM discussed herein primarily function as liquid biopsy biomarkers and signaling molecules rather than as active, colonizing agents.

2. Sources and composition of recirculating microorganisms

For a long time, the academic community has generally regarded blood as an 'absolutely sterile' closed system, where the detection of any microorganisms was equated with severe infection. However, with the rapid advancement of sensitive technologies such as Next-Generation Sequencing (NGS), this classical notion is gradually being rewritten. In 2001, Nikkari *et al* (4) first amplified bacterial DNA in the blood of healthy subjects. Subsequently, Sciarra *et al* (5) and others further confirmed that nucleic acids from archaea, fungi, and viruses could also be stably detected in healthy individuals. A series of findings reveal the existence of a circulating microbial ecosystem within the human body that far exceeds traditional understanding. This ecosystem not only colonizes open cavities like the oral cavity and intestines but may also enter the bloodstream through various pathways, forming a dynamic equilibrium and interacting with the host. This discovery lays a solid foundation for subsequent research into the 'blood microbiome'.

Origin: Gut microbiota, oral cavity, and skin-blood transport pathway. Currently, the presence of CM is largely attributed to microbial transfer from other body sites rich in microbiota, primarily the gastrointestinal tract, oral cavity, and skin. This process is often triggered by compromised structural integrity or functional imbalance of epithelial barriers: for instance, in periodontitis, widened gingival crevicular spaces allow oral microbiota to directly enter microvessels; whereas 'leaky gut' occurs due to downregulation of tight junction proteins and thinning of the mucus layer, enabling trans-epithelial migration of intestinal commensals and their metabolites (6). Once in the bloodstream, bacterial components, including cell wall fragments or cell wall components (lipopolysaccharides, peptidoglycans, flagellar proteins) along with secondary metabolites (short-chain fatty acids and bile acids) circulate systemically, creating a low-grade systemic inflammatory response that provides sustained signaling for microenvironmental remodeling in distant organs (7).

Composition: Reflecting but distinct from gut microbiota. While CM composition mirrors the gut microbiota to some extent, significant alterations occur during translocation. The total human gut microbiota population can reach $4x10^{13}$ CFU, with 97% colonizing the colon. Archaea and eukaryotes (including fungi) constitute only 0.1-1% (8). However, CM represent only a highly selected fraction of these organisms that successfully translocate and are detectable in blood. Notably, the blood microbiome is dominated by the *Proteobacteria phylum*, often reaching relative abundances of 85-90%, exhibiting marked enrichment compared to their relative abundance in the gut, while *Firmicutes*, and *Bacteroidetes* are underrepresented, suggesting selective pressure during translocation (6). However, it is critical to acknowledge that *Proteobacterial* dominance is also the hallmark signature of reagent contamination ('kitome') in low-biomass samples, particularly from *Pseudomonas* and *Acinetobacter* species commonly found in DNA extraction kits and laboratory water. Therefore, whether this dominance reflects true biological translocation or technical artifact remains an active subject of debate and rigorous contamination control. Goraya *et al* (9) further noted that the composition and abundance of the CM exhibit significant heterogeneity across different diseases and across stages of the same disease, reflecting not merely gut dysbiosis but also disease-specific selective pressures in the bloodstream. This provides a new perspective for elucidating the causal role of the CM in disease progression.

3. Circulating microbial detection

Compared to the vast genetic background of the host, the biomass of commensal microorganisms is extremely low, making their precise capture and qualitative/quantitative analysis highly challenging. With the advancement of highly sensitive genetic technologies, methods such as 16S rRNA sequencing, metagenomic sequencing, and intergenic spacer analysis (IS-pro) have become key analytical tools for studying prokaryotes, viruses, fungi, and other microorganisms (Table I).

16S rRNA sequencing is a widely adopted method for analyzing bacteria and archaea (10). This molecular marker combines conserved regions (reflecting species affinity) with highly variable regions (defining species differences), enabling rapid and cost-effective characterization of circulating microbial DNA composition in low-biomass samples like tissues and blood. De Oliveira *et al* (11) utilized high-throughput sequencing of the V3-V4 region to identify an enrichment of colibactin-producing *Escherichia coli* in right-sided colorectal cancer lesions. The study hypothesizes that such bacteria may suppress antigen presentation by reshaping the local glycerophospholipid microenvironment, thereby reducing tumor immunogenicity. However, this conclusion remains a preliminary hypothesis: On one hand, colibactin production is believed to be confined to the tumor microenvironment, lacking direct functional evidence establishing a causal link

Table I. Comparison of methods for detecting CMS.

Characteristic	Testing method		
	16S rRNA sequencing	Metagenomic sequencing	IS-pro technology
Resolution	Genus/Species level	Strain level	Species level
Primer-dependent	Yes (conservative zone + variable zone)	No	Yes (phylum-specific primers)
Testing scope	Bacteria and archaea	Bacteria, archaea, fungi and viruses	Bacteria
Cost	Low	High	Moderate
Turnaround time	1-2 days	4-7 days	3-6 h
Contamination risk	High (PCR amplification preference)	Moderate (host DNA interference)	Moderate
Primary contamination categories	*Pseudomonas*, *Acinetobacter*, *Bacillus*.	*Cutibacterium*, *Pseudomonas* (kit residue); host DNA (>90% of reads)	*Staphylococcus*, *Streptococcus* (skin microbiota)
Sources of contamination	DNA extraction kit (kitome), PCR reagents, laboratory environment, index hopping	DNA extraction kit, host DNA contamination, environmental microorganisms, batch effects	PCR reagents, laboratory environment, operator skin microbiota
Recommended controls	Negative extraction control, PCR negative control, mock community, batch control	Negative extraction control, positive control, batch control, internal reference control	Negative control, standardized strain control, operator control

CMS, circulating microbial signatures; 16S rRNA, 16S ribosomal RNA; IS-pro, intergenic spacer analysis.

to immune evasion *in vivo* (or its presence in the circulating microbial fraction). On the other hand, 16S rRNA sequencing itself is constrained by primer specificity, making it difficult to distinguish closely related species and prone to PCR amplification bias that may overestimate diversity. Nevertheless, this study provides important clues for exploring the potential regulatory mechanisms of CM components on the host microenvironment, while also highlighting the ongoing technical challenges associated with current CM detection methods based on 16S rRNA sequencing (12).

Metagenomic sequencing (also known as shotgun sequencing) involves off-target sequencing of all genomes, enabling simultaneous analysis of both the species composition and functional gene profiles of the CM (13). Chen *et al* (14) combined fecal metagenomics with metabolomics, revealing that *Fusobacterium nucleatum* and *Bifidobacterium longum* accelerate or inhibit colorectal cancer progression via pro-inflammatory metabolites and short-chain fatty acid pathways, respectively, visually demonstrating the causal chain of the 'microbe-metabolite-tumor' axis. Compared to 16S rRNA sequencing, metagenomics is not limited by primers and can capture non-bacterial members such as fungi, viruses, and mycoplasmas. It achieves strain-level resolution, yielding more accurate results. However, this approach requires detecting all gene sequences (including those from normal host cells and tumor cells), making it relatively time-consuming, complex, and costly (15).

IS-pro technology targets the 16S-23S rDNA intergenic spacer region across bacterial species. This fragment exhibits high variability between species while maintaining high intra-species conservation, serving as a bacterial 'fingerprint' (16). Research indicates that IS-pro enables rapid species-level identification and relative quantification in a single-tube reaction through gate-specific PCR primers coupled with fluorescent labeling. Furthermore, compared to 16S rRNA sequencing, IS-pro accelerates analysis, reduces costs, and maintains equivalent analytical performance, offering a scalable, lightweight solution for rapid CM research (17).

Although the emergence of novel detection technologies has facilitated the detection of circulating microbial signatures (CMS), CMS analysis still faces significant technical challenges due to the extremely low microbial DNA biomass in blood. This low biomass characteristic makes CMS research highly susceptible to multiple sources of contamination, including DNA extraction kits ('kit sets'), PCR reagents, laboratory environments, and sequencing tag jumping (18). Studies indicate that contaminant DNA is prevalent in commonly used DNA extraction kits, with significant variation between batches, potentially obscuring genuine biological signals (19).

To ensure reliable interpretation of CMS data, multiple stringent controls must be implemented: i) Negative extraction controls and PCR negative controls; ii) batch randomization and calibration; iii) computational decontamination (removing contaminants like *Pseudomonas* and *Bacillus* detected in negative controls); iv) quantitative PCR measurement of bacterial DNA load to distinguish true signals from background contamination (18).

These limitations significantly impact the interpretation of CMS studies. Research lacking adequate controls or decontamination steps may misidentify kit contaminants as disease biomarkers. Furthermore, variations in blood collection tube

types, storage conditions, and extraction protocols introduce batch effects that interfere with cross-study comparisons. Therefore, caution is warranted when interpreting CMS findings, and standardized contamination control protocols are urgently needed in this field.

4. The link between circulating microorganisms and gastrointestinal tumors

In recent years, as the aforementioned detection technologies have transformed CM into quantifiable liquid biopsy markers, it has been confirmed that CM may be associated with synergistically regulating host immunity, inflammatory signaling, and tumor microenvironment remodeling through bacterial structures, nucleic acid fragments, and metabolic small molecules. The following sections will systematically examine the pivotal role of CM in gastrointestinal tumorigenesis, progression, and outcomes from two dimensions: 'Diversity disparities' and 'oncogenic mechanisms'. This analysis aims to provide novel strategic footholds for early diagnosis, precision therapy, and prognostic assessment.

Diversity differences: From gut dysbiosis to circulating signals. The composition and diversity of the gut microbiota in gastrointestinal cancer patients undergo significant remodeling, contrasting sharply with healthy individuals. Colorectal cancer (CRC) is a malignant tumor originating in the colon or rectum, classified as colon cancer or rectal cancer based on its site of origin (20). Compared to control patients, colorectal cancer patients frequently exhibit increased levels of pro-inflammatory bacterial species, including *Clostridium difficile* and *Bacteroides fragilis*; conversely, samples from control patients typically show greater abundance of beneficial bacteria such as *Bifidobacterium* and *Bacteroides* species (21).

CM as liquid biopsy markers. Critically, these gut dysbiotic signatures translate into detectable CM alterations. Giacconi *et al* (19) conducted a case-control study analyzing plasma samples from 50 CRC patients and 40 healthy controls, demonstrating significantly elevated bacterial DNA loads in the circulation of CRC patients compared to controls ($P<0.001$). Their analysis revealed distinct microbial signatures, including increased abundance of oral-originating taxa such as *Cutibacterium* and *Sphingomonas*, that could discriminate early-stage CRC from healthy individuals with high accuracy (AUC=0.92). These findings suggest that translocated microbial DNA in circulation reflects underlying gut dysbiosis and tumor-associated microbial shifts, supporting the theoretical potential of CM as liquid biopsy biomarkers for CRC detection, pending standardized validation.

Gastric cancer exemplifies the interaction between CM dysbiosis and host epithelial cells. *Helicobacter pylori* (Hp) is the most extensively documented risk factor for gastric cancer, accounting for approximately 65-80% of all cases. It may be associated with driving patients' progression from atrophic gastritis to intestinal metaplasia (22). It can also enter the bloodstream through mucosal lesions to form CM, whose DNA and antigenic components can be detected in the patient's peripheral blood. Beyond Hp, the gut microbiota of gastric cancer patients exhibits enrichment of pro-inflammatory bacteria such as *Proteobacteria* and *Spirochaetes*, alongside reduced levels of beneficial bacteria like *Firmicutes* (butyrate producers) and *Bacteroidetes* (bile acid degraders) (23). Hu *et al* (24) demonstrated that bovine anti-*H. pylori* antibodies effectively clear *H. pylori* infections in individuals with blood type O. They also reported that other spirochetes (e.g., *Helicobacter heilmannii*) can colonize the human stomach alongside *H. pylori*. This dysbiosis is associated with intestinal barrier disruption, inflammatory responses, and tumor-promoting microenvironmental changes.

Microbiome dysbiosis-leaky gut-inflammation axis: Pathogenic mechanisms of CM disruption. Dysbiosis may compromise the intestinal barrier through mechanisms such as downregulating tight junction proteins, disrupting the mucus layer, and causing immune dysregulation, potentially facilitating bacterial translocation into the bloodstream. Notably, barrier disruption permits the translocation of diverse, as-yet-uncharacterized microbial species and their products; consequently, while barrier dysfunction and inflammation are plausible contributors to colorectal carcinogenesis, ascribing tumor-promoting effects to specific taxa or discrete mechanisms remains challenging at this stage. Zhang *et al*'s circadian rhythm disruption (CRD) mouse model demonstrated that 21 days of continuous light exposure increased intestinal permeability by 114.7%, significantly elevated plasma FITC-dextran levels, and induced a reduction in *Prevotellaceae* alongside an increase in *Bacteroidaceae*, forming a vicious cycle of 'dysbiosis-leaky gut-circulating microbial dysregulation' (25). Lipopolysaccharide (LPS) derived from intestinal Gram-negative bacteria enters the liver via the portal vein and is normally cleared by Kupffer cells. When clearance efficiency declines, LPS translocate into the bloodstream, triggering metabolic endotoxemia. This activates the TLR4-NF-κB pathway, promoting the release of pro-inflammatory factors such as IL-1β, IL-6, and TNF-α, leading to low-grade chronic inflammation and significantly increasing disease risk (26).

5. Mechanistic studies of CM and gastrointestinal tumors

The mechanisms by which circulating microorganisms contribute to gastrointestinal tumorigenesis involve complex interactions between microbial components and host signaling pathways (Fig. 1). Proposed mechanisms implicating CM in chronic inflammation and immune evasion lies in gut microbiota dysbiosis but manifests systemically through translocated components. PAMPs, namely LPS and flagellar proteins from gram-negative gut bacteria, first breach the intestinal barrier via paracellular or transcellular routes, entering the bloodstream as CM components. Once in circulation, these PAMPs bind with high affinity to Toll-like receptor 4 (TLR4) on distant immune cells and endothelial cells, activating systemic rather than localized inflammatory responses (27).

Upon binding to TLR4 on intestinal epithelial or immune cells, LPS rapidly activates dual signaling pathways, NF-κB and MAPK, via the MyD88 adaptor protein, continuously amplifying proinflammatory signals such as IL-6, IL-1β, and TNF-α. This systemic inflammation, driven by circulating microbial components (such as PAMPs and metabolites) rather

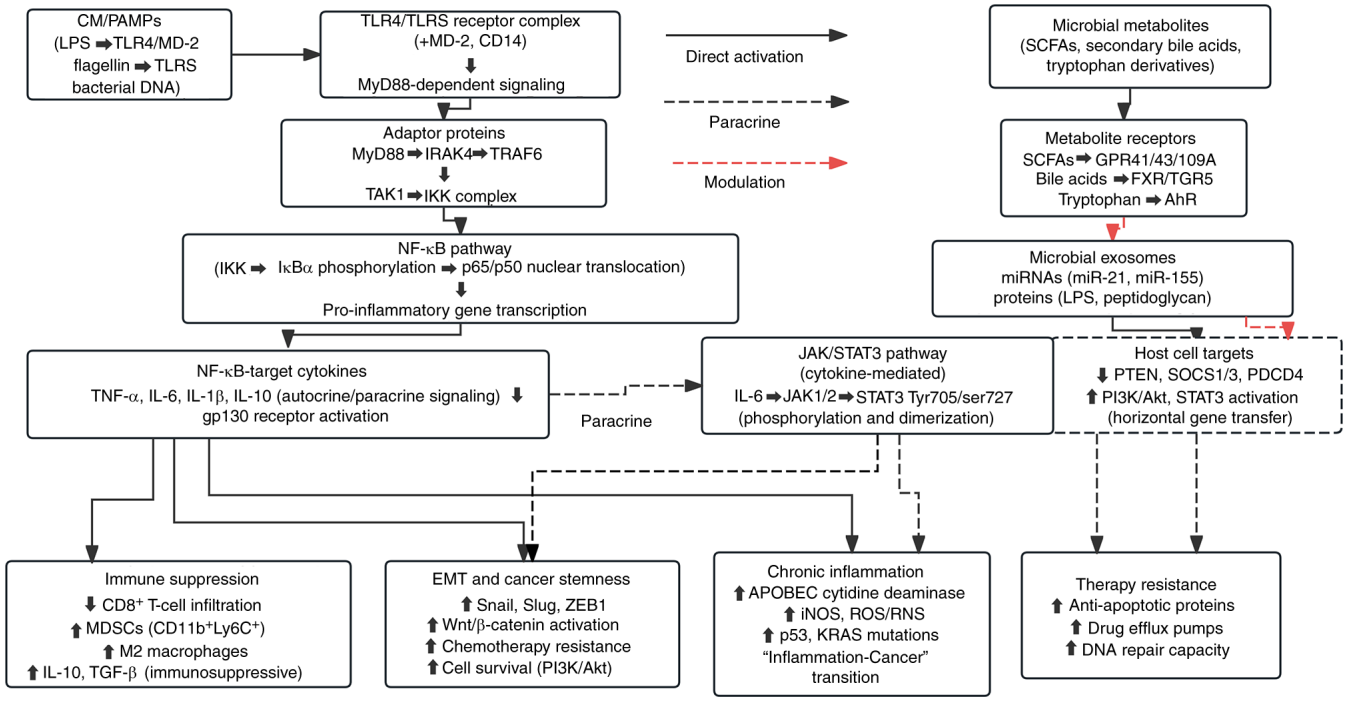

Figure 1. Mechanistic pathways of CMS in gastrointestinal tumorigenesis. CMS, circulating microbial signatures. CMS, circulating microbial signatures; CM, circulating microorganisms; PAMPs, pathogen-associated molecular patterns; LPS, lipopolysaccharide; TLR, Toll-like receptor; IRAK4, IL-1 receptor-associated kinase 4; TRAF6, TNF receptor-associated factor 6; TAK1, TGF-β-activated kinase 1; IKK, IκB kinase; STAT3, signal transducer and activator of transcription 3; APOBEC, apolipoprotein B mRNA-editing enzyme catalytic; iNOS, inducible nitric oxide synthase; ROS, reactive oxygen species; RNS, reactive nitrogen species; MDSCs, myeloid-derived suppressor cells; EMT, epithelial-mesenchymal transition; ZEB1, zinc finger E-box binding homeobox 1; SCFAs, short-chain fatty acids; FXR, farnesoid X receptor; TGR5, Takeda G protein-coupled receptor 5; AhR, aryl hydrocarbon receptor; miR, microRNA; PDCD4, programmed cell death 4.

than localized gut bacteria, leads to recruiting CD11b+ Ly6C+ myeloid-derived suppressor cells myeloid-derived suppressor cells, inducing macrophage polarization toward the M2 phenotype, and secreting IL-10 and TGF-β. This may directly inhibit CD8+ T cells infiltration, potentially weakening the first line of immune defense (28). Furthermore, the chronic inflammatory environment upregulates apolipoprotein B mRNA editing enzyme catalyzing polypeptide (APOBEC) cytidine deaminase and nitric oxide synthase, driving the accumulation of mutations in key genes like p53. This may promote the 'inflammation-to-cancer' transition and early immune evasion (27).

Moreover, PAMPs can trigger signal transducer and activator of transcription 3 (STAT3) serine phosphorylation via the TLR4/TLR5-JAK2 axis, driving epithelial-mesenchymal transition, maintenance of cellular stemness, and the formation of drug-resistant phenotypes. Balic *et al* (29) confirmed that this non-canonical STAT3 activation is a core node in TLR4-mediated glycolytic reprogramming and macrophage polarization. Extensive crosstalk exists between the Wnt and STAT3 pathways; microbe-mediated STAT3 activation indirectly enhances Wnt signaling, further boosting chemotherapy tolerance (30). Concurrently, microbial metabolites or LPS-induced inflammatory factors like TNF-α inhibit apoptosis by activating the PI3K/Akt pathway, thereby enhancing tumor cell survival and resistance to chemotherapeutic agents (31).

Beyond the bacteria themselves, secondary bile acids, short-chain fatty acids, and microbial exosomes in CM serve as critical chemical messengers in the 'inflammation-to-cancer' evolution. Gao *et al* (32) demonstrated that high concentrations of deoxycholic acid (DCA), generated from hepatic primary bile acids via 7α-dehydroxylation, act as DNA-breaking agents and epigenetic disruptors. DCA inhibits DNA repair enzymes in intestinal epithelial cells, increases alkylating agent-induced mutation rates, and amplifies NF-κB inflammatory signaling, significantly elevating gastrointestinal tumor risk. Meanwhile, reduced butyrate-producing bacteria *in vivo* cause insufficient β-oxidation energy supply in intestinal epithelium, while abnormal increases in propionic acid and isovaleric acid enhance tumor cell migration and metastasis via the GPR43-PI3K axis (33). Furthermore, circulating microbial components, particularly PAMPs, induce host cells to release exosomes rich in specific miRNAs and proteins. Ponton-Almodovar *et al* (34) propose that these exosomes, once internalized by tumor cells, sustain activation of PI3K/Akt and STAT3 pathways by downregulating tumor suppressors like PTEN and SOCS6, thereby synergistically driving metastasis and chemotherapy resistance.

In summary, CM have been implicated in contributing to a self-reinforcing oncogenic network through TLR-inflammation-mutation-pathway activation-metabolic reprogramming-exosome remote regulation, potentially spanning the initiation, progression, and resistance of gastrointestinal tumors.

It is important to note that the mechanistic pathways discussed above, including TLR-NF-κB, STAT3, Wnt, and PI3K/Akt signaling, have been primarily established through studies of gut-resident or intratumoral microbiota rather than circulating microbial components per se. The extrapolation of

these mechanisms to CM is based on the shared molecular structures (e.g., LPS, flagellin) between tissue-resident and circulating microbes. However, whether CM activate identical signaling nodes with comparable kinetics and potency as their tissue-bound counterparts requires direct experimental validation in future studies.

6. Circulating microbes shape epigenetic memory: The long-term legacy of inflammation

Prolonged exposure to circulating microbial components can induce epigenetic reprogramming, driving persistent alterations in gene expression. This process encompasses abnormal changes in DNA methylation patterns, chromatin structural remodeling, and sustained activation of inflammatory transcription programs. The resulting epigenetic imprints persist long after the initial stimulus has completely subsided, driving fundamental shifts in cellular function.

At the DNA methylation level, chronic activation of the TLR4 and NF-κB signaling pathways can recruit DNA methyltransferases to specific genomic regions, leading to hypermethylation of tumor suppressor gene promoters (8). For instance, prolonged exposure to LPS in the human microenvironment induces significantly elevated methylation levels in promoter regions such as p16, thereby causing gene silencing and impairing DNA damage repair capacity (35).

Histone modifications also represent a core component in inflammation-mediated epigenetic alterations. Key transcription factors like NF-κB and signal transducer and activator of STAT3 recruit histone acetyltransferases to gene enhancer regions, maintaining sustained activity of proliferation-related genes and establishing a stable pro-tumor transcriptional environment. Concurrently, this 'open' chromatin conformation possesses epigenetic memory properties, allowing the state to persist long-term even after pathogenic microorganisms are completely cleared, without requiring continuous external stimulation (36).

Microbial metabolites in the circulation can also participate in gene expression reprogramming by directly regulating the activity of epigenetic enzymes. As a natural inhibitor of histone deacetylases (HDACs), reduced circulating levels of butyrate, commonly observed in patients with advanced gastrointestinal tumors, lead to dysregulated HDAC hyperactivation. This triggers abnormal chromatin condensation and silences key DNA repair genes (37).

These multi-layered epigenetic alterations provide a molecular explanation for a key clinical observation: even after successful early eradication of pathogenic bacteria (such as *Helicobacter pylori*), the host's cancer risk does not immediately decline (38). Because inflammation-induced epigenetic 'memory' is firmly established in target tissue cells, this intrinsic driver of malignant transformation persists and operates autonomously. Even after the original circulating microbial components have completely disappeared, the organism inevitably continues progressing along the inflammation-to-cancer conversion trajectory.

7. Clinical translation of CMS and validation requirements

Currently, multiple studies have explored the potential of CMS as non-invasive liquid biopsies for gastrointestinal tumors. In the field of CRC, circulating bacterial DNA levels in CRC patients are significantly higher than in healthy controls and positively correlate with tumor burden (19). Microbiome-based cfDNA classifiers achieve 90% accuracy in distinguishing primary hepatocellular carcinoma from metastatic CRC (39). In gastric cancer, detection of *Helicobacter pylori* DNA and antigen components in peripheral blood provides direct evidence of bacterial translocation triggered by mucosal lesions, effectively supplementing diagnostic information (6). Furthermore, the association between CMS and postoperative recurrence risk in lung cancer suggests broad prospects for this approach across cancer types (7).

However, its clinical translation still faces significant challenges. Existing studies are predominantly single-center designs with limited sample sizes and lack prospective validation in independent cohorts. Additionally, the absence of universally accepted negative control standards and protocols for calculating decontamination processes complicates cross-study comparisons. More importantly, the precise origin of circulating microbial DNA (intestinal, oral, or other sites) and its functional relationship with the live microbial community remain to be elucidated. Future large-scale, multicenter prospective studies are urgently needed. These should establish the clinical utility of circulating microbiome as a liquid biopsy tool through rigorous contamination control, standardized methodologies, and integration with established clinical biomarkers.

8. Application of circulating microorganisms in gastrointestinal tumor therapy

Currently, surgery combined with chemoradiotherapy remains the standard treatment regimen for gastrointestinal tumors. Recent studies on circulating microorganisms have revealed its dual 'oncogenic-suppressive' role in tumor initiation and progression. By interacting with host immunity and metabolism, CM can either enhance or diminish treatment response, positioning it as a potential key target for precision interventions. Furthermore, emerging strategies such as engineered strain immunotherapy, probiotic interventions, and fecal microbiota transplantation further highlight the role of CM in exploring novel cancer treatments.

CM and chemotherapy. Chemotherapy remains one of the core treatment modalities for advanced gastrointestinal tumors, yet the emergence of chemotherapy resistance significantly shortens patient survival (40). Recent evidence indicates that microbial enzymes detected in circulation (likely originating from translocated bacteria or circulating microbial fragments) alter the activity of chemotherapeutic agents, positioning them as both 'accomplices' and 'assistants' in tumor treatment (41). On one hand, enzymes associated with certain CMs inactivate drugs by altering their structures; for example, Vibrio gamma-deoxythymidine deaminase inactivates gemcitabine, inducing resistance in pancreatic ductal adenocarcinoma, an effect reversible by ciprofloxacin (42). Similarly, bacterial β-glucuronidase converts the inert metabolite of irinotecan into its active form, triggering dose-limiting diarrhea (43).

Conversely, multi-omics experiments by the Iida team confirmed that probiotics like *Lactobacillus* acidophilus

activate tumor-associated macrophages and dendritic cells via the TLR-MyD88-NF-κB axis. This induces sustained release of high concentrations of reactive oxygen species (ROS), enhancing DNA damage in tumor cells while blocking repair and transcription pathways, thereby boosting the efficacy of the anticancer drug oxaliplatin (44). The dual nature of CM, both antagonizing and enhancing chemotherapy effects, underscores the necessity for further investigation. Only by thoroughly understanding the specific drug-microbiome interactions can precise microbial intervention strategies be designed to optimize therapeutic outcomes.

CM and radiation therapy. Radiotherapy relies on ionizing radiation to exert cytotoxic effects on tumor cells, serving as another treatment modality for mid-to-late stage tumors. Recent studies indicate that commensal microorganisms such as CM significantly influence radiotherapy efficacy and prognosis (45). Selective elimination of Gram-positive bacteria via oral vancomycin enhances antitumor activity during radiotherapy by facilitating cross-presentation of tumor-associated antigens to CD8+ T cells and promoting IFN-γ secretion (46). Conversely, broad-spectrum antibiotic mixtures, while eliminating symbiotic bacteria, promote the expansion of symbiotic fungi, thereby diminishing radiosensitivity (47). In the same year, Dong *et al* (45) demonstrated significantly improved radiotherapy outcomes after using metronidazole to specifically eliminate *Clostridium difficile*. Furthermore, researchers discovered that tumor *lactic acid bacteria* producing L-lactic acid can induce radiation resistance in tumor cells through metabolic remodeling (48). Collectively, these findings suggest that optimizing disease-specific and personalized probiotic combinations and applications will be an essential future research direction, building upon improvements in radiotherapy efficacy and reduction of radiation-induced damage.

CM and novel engineering therapies. In recent years, fecal microbiota transplantation (FMT) has emerged as a novel approach for reshaping the gut microbiome, demonstrating significant advantages in treating gastrointestinal diseases. FMT involves transferring the fecal microbiota from a healthy donor into a patient's gut to restore microbial balance, with demonstrated efficacy in treating conditions such as recurrent *Clostridioides difficile* infection and ulcerative colitis (49,50). In oncology, FMT can recruit antigen-presenting cells like dendritic cells into the tumor microenvironment, reshape innate and adaptive immunity, and exert antitumor effects through microbial metabolism (51). Davar *et al* (52) demonstrated that FMT from responder donors reversed immune therapy efficacy in some of 15 advanced melanoma patients resistant to anti-PD-1 therapy, confirming FMT's potential for cancer prevention and treatment. However, the mechanisms of action, ethical guidelines, and optimal protocols for FMT require further clarification through high-quality randomized controlled trials.

Probiotics and prebiotics represent another hotspot in microbiome interventions. Probiotics are live beneficial microorganisms that activate macrophages and induce antitumor factors such as TNF-α, IFN-γ, IL-12, and NO. Prebiotics, conversely, are substrates utilized by these beneficial bacteria, selectively promoting the proliferation of beneficial commensal flora (53). Zheng *et al* (54) demonstrated in a rat gastric cancer postoperative model that composite probiotics reduce inflammation, downregulate intestinal permeability signals, and restore microbial community structure. However, clinical translation of probiotics faces challenges including short shelf life and quality variability due to production and storage differences. Standardized protocols and long-term follow-up data are urgently needed to support their integration into frontline cancer treatment.

Overall, FMT and probiotic interventions restore barrier function by reshaping the gut microbiome, reducing bacterial translocation and the entry of pro-tumor factors into the bloodstream. Simultaneously, they competitively inhibit pathogenic bacteria, block carcinogenic components from entering the bloodstream, promote the systemic release of beneficial metabolites, stabilize the circulating microbiome, indirectly regulate cell membrane composition and function, and drive the body toward restoring a healthy steady state.

9. Conclusion

In recent years, with the iterative development of high-sensitivity genetic technologies, CM has evolved from a 'detection noise' to a core liquid biomarker potentially associated with driving the occurrence, progression, and efficacy regulation of gastrointestinal tumors. This paper examines CM research progress in gastrointestinal tumors across four dimensions, revealing a 'gut dysbiosis-CM translocation-systemic tumor microenvironment remodeling' axis that offers novel perspectives for non-invasive diagnosis.

However, as reviewed herein, current evidence remains predominantly preclinical or derived from small-scale retrospective cohorts. Future research should focus on the following pathways: First, integrate individual patient data on metagenomics, metabolomics, and immune phenotypes; second, employ machine learning algorithms to eliminate contamination signals from kits and laboratories, thereby identifying microbial biomarkers with diagnostic value; finally, validate through multicenter prospective cohort studies whether these biomarkers exhibit a direct causal relationship with tumor development, rather than merely being associated phenomena. Should standardized protocols and rigorous clinical validation be achieved, real-time monitoring of CM dynamics could eventually contribute to the development of 'liquid microbiome precision medicine'. Currently, however, CM-based diagnostics remain in the proof-of-concept phase, requiring substantial methodological refinement before clinical implementation.

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Authors' contributions

YY and YD conceptualized the study. YY and YR conducted the investigation. YY wrote the original draft. TM, SJ, YR, JA and YD reviewed and edited the manuscript. SJ and JA prepared the figures. YD supervised the study. Data authentication is not applicable. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

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Competing interests

The authors declare that they have no competing interests.

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