Open Medicine Journal
DOI: 10.2174/0118742203388914250710052206, 2025, 12, e18742203388914
ISSN: 1874-2203

REVIEW ARTICLE OPEN ACCESS

Role of Microbiota in Pathogenesis of Gastrointestinal Cancers

Abbes Belkhiri[1] and Nataliya Pidkovka[2,*]

[1]*Baptist University College of Osteopathic Medicine, Baptist Health Sciences University, Memphis, TN*
[2]*Department of Health Science, South College, Nashville, TN*

Abstract:
Gastrointestinal malignancies are a leading cause of cancer-related deaths and are linked to changes in microbiota composition. A body of accumulating evidence indicates that microbial dysbiosis plays a crucial role in neoplastic transformation and oncogenesis of the digestive system organs. This includes modulation of immune responses, alteration of the tumor microenvironment, and metabolic activities of gut bacteria, such as the production of short-chain fatty acids, bile acids, toxins, and genotoxins. Several clinical trials have recently been initiated to test fecal microbiota transplantation for improving the efficacy of immunotherapies and conventional chemotherapeutics in gastrointestinal cancers. This review summarizes progress in understanding the mechanisms driving microbiota's role in gastrointestinal tumorigenesis and how microbiota influences therapy response and discusses microbiota-based potential therapeutic applications.

Keywords: Gastrointestinal cancers, Gut microbiota, Immunotherapy, Immune checkpoint inhibitors, Tumor microenvironment, Fecal microbiota transplantation, Bacterial genotoxins.

© 2025 The Author(s). Published by Bentham Science.
This is an open access article distributed under the terms of the Creative Commons Attribution 4.0 International Public License (CC-BY 4.0), a copy of which is available at: https://creativecommons.org/licenses/by/4.0/legalcode. This license permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

*Address correspondence to this author Department of Health Science, South College, Nashville, TN;
E-mail: npidkovka@south.edu

Cite as: Belkhiri A, Pidkovka N. Role of Microbiota in Pathogenesis of Gastrointestinal Cancers. Open Med J, 2025; 12: e18742203388914. http://dx.doi.org/10.2174/0118742203388914250710052206

Received: January 28, 2025
Revised: April 23, 2025
Accepted: May 02, 2025
Published: July 18, 2025

Send Orders for Reprints to
reprints@benthamscience.net

1. INTRODUCTION

The normal physiology of the gastrointestinal (GI) tract involves a close relationship with the microorganisms populating it, collectively known as the microbiota. Disturbances in the homeostasis of the digestive system, such as those caused by GI malignancies, are possibly linked to alterations in microbiota composition and abundance. Notably, neoplasia of the GI tract remains a leading cause of cancer-related deaths. In 2022, colorectal, liver, gastric, pancreatic, and esophageal cancers were the second, third, fifth, sixth, and seventh leading causes of cancer-related deaths in the USA, respectively [1, 2]. Accumulating evidence suggests that microbiota play an essential role in GI neoplastic transformation. The central objective of this review is to summarize the progress made in understanding the underlying mechanisms defining the role of microbiota and its metabolic products in GI tumorigenesis and highlight the potential therapeutic applications of microbiota.

Despite these advances in understanding the microbiome and GI cancer pathophysiology, most studies remain descriptive, primarily focused on the imbalanced bacterial ecosystem, known as dysbiosis, within the context of cancer. Alterations of microbiota populating the GI tract were reported in patients with malignancies of the esophagus [3], liver [4], pancreas [5], and colorectal region [6, 7]. The major challenge in the field lies in the absence of a specific qualitative definition of 'normal' versus 'abnormal' microbiota [8], contributing to individual variability in drug response, as the GI microbiome is highly dynamic. Findings from studies on

microbiome dynamics in cancer reveal potential biomarkers for treatment response, suggesting that modulating microbiota could enhance therapeutic efficacy in a personalized approach.

2. MATERIALS AND METHODS

To explore the emerging role of gut microbiota in the pathogenesis and treatment of GI cancers, a focused literature search was performed using PubMed and ClinicalTrials.gov. Given the novelty of the subject and the limited availability of comprehensive data, the search strategy was intentionally broad and encompassed peer-reviewed studies and clinical trials published in English between 1984 and December 2024. The search utilized a combination of relevant keywords and MeSH terms, including gastrointestinal cancers, colorectal cancer, gastric cancer, gut microbiota, intestinal microbiome, immunotherapy, immune checkpoint inhibitors, tumor microenvironment, fecal microbiota transplantation [FMT], and bacterial genotoxins. Filters were applied to restrict results to human or in vivo studies, original peer-reviewed articles, and registered interventional and observational trials.

Studies were considered eligible if they focused on the microbiota's contribution to GI tumorigenesis or its interaction with therapeutic approaches and provided mechanistic, clinical, or translational insights. Only English-language publications with accessible full texts were included. Studies were excluded if they were editorials, opinion pieces, abstracts without complete data, or if they lacked relevance to GI cancers or microbiota-related mechanisms. In vitro-only studies were also excluded unless they offered directly applicable mechanistic insights into in vivo phenomena.

All retrieved citations were imported into EndNote for deduplication and reference management. Titles and abstracts were independently screened for relevance by the authors, followed by a full-text review of eligible articles. Final study inclusion was determined through discussion and consensus. From each included study, key data were extracted and analyzed, including study design and population, the specific GI cancer subtype investigated, treatment context, microbial taxa or metabolites described, immunological mechanisms such as checkpoint inhibition, and therapeutic interventions including antibiotic therapy, fecal microbiota transplantation, or probiotics.

3. MODULATION OF RESPONSES TO CANCER THERAPY

Immune checkpoint inhibitors [ICIs], such as nivolumab and pembrolizumab, are novel and increasingly effective therapies for several types of neoplasia, primarily melanoma [9, 10]non-small-cell lung cancer [NSCLC] [11], kidney cancer [12], and recently upper GI and biliary cancers [13]. The targets for these therapies include programmed death protein 1 [PD-1] and cytotoxic T-lymphocyte-associated protein 4 [CTLA-4] pathways, which are essential for preventing autoimmunity and regulating anti-tumor immune responses. PD-1 binds to its ligands, PD-L1 [also known as CD274] and PD-L2 [CD274], which are frequently expressed on cancer cells, including GI cancers [14-16]. This binding leads to a decrease in T-cell activity, allowing cancer cells to evade the immune response. Similarly, CTLA-4 acts as a negative regulator of T-cell activation [17] by binding to its ligands, CD80 or CD86, on the surface of antigen-presenting cells [18]. Remarkably, Routy et al. in 2018 demonstrated an association between antibiotic consumption and a poor response to immunotherapeutic PD-1 blockade in patients with lung and kidney cancers. Fecal microbiota transplantation [FMT] from ICIs-sensitive cancer patients into antibiotic-treated mice rescued the anti-cancer effects of PD-1 blockade, in contrast to FMT from ICIs-resistant patients [19]. Metagenomic data from cancer patient fecal samples and FMT experiments in germ-free mice models revealed associations between PD-1/interleukin-12-dependent clinical responses to ICIs and the relative loads of the bacterium *Akkermansia muciniphila* [19]. Similarly, a significant association was observed between gut microbiota composition and the clinical response in melanoma pre-clinical models [20] and patients receiving PD-1 inhibitors [21, 22]. Bacterial species found in greater abundance in cancer patients sensitive to the treatment included *Bifidobacterium longum, Collinsella aerofaciens, and Enterococcus faecium* [22].

It is highly possible that resident microbiota can affect patient responses to cancer immunotherapy. This effect is probably more evident in GI malignancies, as gut commensals are a necessary part of the homeostasis of the digestive system. However, there is conflicting evidence regarding the effect of ICIs on patients with GI cancer [23, 24], while the potential involvement of the PD-1 pathway in GI carcinogenesis is conceivable [15, 25, 26]. In a study conducted at Beijing Cancer Hospital, levels of circulating PD-L1 were shown to be significantly elevated in the serum of advanced gastric cancer [GC] patients compared to healthy controls [16]. The expression of PD-L1 was significantly correlated with the degree of tumor differentiation and lymph node metastasis [16]. Furthermore, the amplification of PD-L1 and PD-L2 genes was detected in the molecular profiles of 295 primary gastric adenocarcinomas analyzed as part of the Cancer Genome Atlas [TCGA] project [14]. Elevated PD-1 expression was also demonstrated in tissue microarrays of esophagectomy specimens collected from locally advanced esophageal squamous cell carcinoma [ESCC] patients [27]. A meta-analysis of survival data for patient subgroups with low PD-L1 expression from clinical trials comparing ICIs with chemotherapy in ESCC revealed no significant survival benefit for immunotherapy-based treatments as the initial intervention, compared to chemotherapy alone, in the subgroup with a tumor proportion score below 1% [28]. Thus, the expression of PD-1, PD-L1, and PD-L2 potentially could serve as promising predictive biomarkers for ICIs therapies in GI cancers. However, there is limited data available on the impact of ICIs on patients with GI neoplasms in relation to human gut microbiota, which

exhibits highly differential composition. We summarized the data on the prevalent microbiota of GI cancer patients responsive to ICIs in Table **1**.

Table 1. Gut microbiome of GI cancer patients responsive to the treatment with ICI.

Bacteria	Type of Cancer	Refs.
Alipes, Parabacteroides, Phascolarctobacterium, Collinsella, Ruminiclostridium, Porphyromonas, Butyricimonas, and Fibrobacteraceae	GI	[29]
Prevotella merdae Immunoactis, Lactobacillus salivarius, and *Bacteroides plebeius*	Refractory advanced solid carcinoma	[30]
Prevotella, Ruminococcaceae, and *Lachnospiraceae*	Advanced GI cancer	[31]
Fusobacterium nucleatum	MSS CRC	[32]
Lachnospiraceae bacterium-GAM79, Alistipes sp Marseille-P5997, Ruminococcus calidus, and *Erysipelotichaceae bacterium-GAM147*	Unresectable hepatocellular carcinoma or advanced biliary tract cancer	[33]

In a study of 27 patients with different types of cancer, including 9 individuals with cancers of the digestive system, differences in baseline microbiota and its alteration after anti-PD-1 treatment combined with chemotherapy were detected in patients sensitive to the treatment as compared to non-responders [29]. Particularly, in the fecal samples from patients who responded to the therapy, an increase in *Weissella* abundance was detected at the sixth week of treatment. In non-responders, the therapy was associated with an increase in *Fusobacterium* and *Anaerotruncus* at week twelve [29]. The Authors also determined the prevalence of *Firmcutes* bacteria in a group of patients exhibiting a higher rate of adverse effects, while *Bacteroidetes were* enriched in the samples of individuals without detected adverse effects following anti-PD1 therapy [29]. An increase of *Prevotella/Bacteroides* ratio has been previously linked to a favorable response to anti–PD-1 therapy in advanced-stage GI cancers [31].

Several studies have demonstrated the ability of colorectal *Fusobacteria* to alter *the* tumor microenvironment that could lead to poor clinical outcomes, including colorectal cancer (CRC) growth [34, 35]. Interestingly, *Fusobacterium nucleatum* [*F. nucleatum*] sensitized microsatellite stable (MSS) CRC to anti-PD-1 immunotherapy [32]. In experiments on germ-free humanized mice bearing MSS CRC, FMT from patients with high loads of *F. nucleatum* improved sensitivity to anti-PD-1 treatment compared to *F. nucleatum*-low FMT. Single *F. nucleatum* treatment also enhanced anti-PD-1 efficacy in murine allografts and CD34+-humanized mice bearing MSS CRC [32]. In fact, *Fusobacterium* has been proposed as a microbial carcinogen that promotes the initiation and progression of CRC [35-37]. Multiple studies have demonstrated that patients with CRC who harbor high levels of *F. nucleatum* have poorer survival rates [34]. Furthermore, *Fusobacterium* tends to colonize areas of tumors exhibiting immune and epithelial cell activities that promote cancer progression [38, 39]. Microbiome modulation studies targeting *F. nucleatum* suggest that *Fusobacterium* might affect cancer progression [40, 41] and metastasis [40]. Similar to the strong correlation between *Helicobacter pylori* infection and GC [42, 43], *Fusobacterium* may be an important microbial carcinogen that drives the initiation and progression of CRC.

The idea that FMT with favorable microbiota can overcome resistance to anti-PD-1 inhibitors in advanced unresectable or metastatic solid cancers, particularly GI cancers, was tested in a clinical trial NCT04264975 [30] showing that FMT from anti-PD1-sensitive patients improved clinical response in 6 of 13 patients total, resulting in an objective response rate of 7.7% and a disease control rate of 46.2% [30]. The positive clinical effect correlated with increased levels of cytotoxic T cells [30]. Metagenomic analysis of feces isolated from recipients who received FMT from PD-1-sensitive donors demonstrated the presence of bacteria *Prevotella merdae, Lactobacillus salivarius*, and *Bacteroides plebeius* [30]. In fact, an increase of *Prevotella/Bacteroides* ratio has been linked to a favorable response to anti–PD-1 therapy in advanced-stage GI cancers [31]. The clinical response to anti-PD-1 therapy in patients with hepatobiliary cancers [HBC] was associated with higher abundance of *Lachnospiraceae bacterium-GAM79, Alistipes spp. Marseille-P5997, Ruminococcus calidus*, and *Erysipelotichaceae bacterium-GAM147* than in patients with lower abundance of these bacteria in stool [33]. Gungur at el, suggested that future development of gut microbiome diagnostics or therapeutics should be tailored according to immune checkpoint blockade [ICB] treatment regimen rather than according to cancer type [13]. Recent studies have highlighted the complex interplay between microbiota and chemotherapy efficacy in GI cancers. For instance, the efficacy of banoxantrone [AQ4N] was enhanced, while the efficacy of Gemcitabine and CB1954 was reduced in the presence of *E. coli*, as demonstrated in *in vivo* murine subcutaneous tumor xenograft models of CRC [44]. In contrast, a retrospective analysis of clinical data from 580 patients with respectable or metastatic pancreatic ductal adenocarcinoma [PDAC] who underwent chemotherapy regimens with gemcitabine or 5-fluorouracil [5FU] revealed that antibiotic-associated modulation of the microbiome is linked to better outcomes in patients with metastatic PDAC [45].

Together, these data strongly suggest that manipulating GI microbiota may modulate the efficacy of cancer therapies.

4. INFLAMMATION AND ALTERATION OF THE TUMOR MICROENVIRONMENT

Induction of pro-inflammatory signaling pathways is established as a hallmark of carcinogenesis. Studies on a rat model of esophageal adenocarcinoma [EAC], supported by data from human clinical samples of Barrett's esophagus and EAC, revealed an association between the Toll-like receptor signaling pathway and *E. coli* abundance

[46]. In contrast, another study demonstrated that altering microbiota with antibiotics did not impact the development of EAC in rats [47].

In the insulin-gastrin mouse model of gastric carcinogenesis, gastric colonization with *H. pylori* and intestinal microflora containing *Lactobacillus, Bacteroides*, and *Clostridium* species caused severe inflammation, characterized by elevated mRNA expression of IL11, Ptger4, and TGF-β, as well as the development of spasmolytic polypeptide-expressing metaplasia [48]. In mice, a high-fat diet induced gastric dysbiosis with an increased abundance of *Lactobacillus*. This was accompanied by intestinal metaplasia, increased levels of intracellular β-catenin and gastric leptin, and phosphorylation of the leptin receptor and STAT3 [49]. The analysis of single-cell RNA sequencing data of human GC tissues revealed that *H. pylori* infection leads to the upregulation of the uridine phosphorylase 1 [UPP1] gene expression through the NF-κB pathway and activation of the Macrophage Migration Inhibitory Factor pathway in the tumor microenvironment TME [50]. It has been proposed that intestinal dysbiosis can result in the propagation of specific bacteria that drive colon carcinogenesis through chronic inflammation or local immunosuppression mechanisms [51, 52]. T cell infiltration of solid tumors is linked to favorable patient outcomes [20, 53]But the mechanisms behind the varying individual immune responses remain poorly understood. Similarly, the impact of normal microbiota on the host immune system is not well understood yet. A direct relationship has been identified between the presence of specific bacteria in the intestinal microbiota and T cell development and differentiation [54, 55]. The intestinal microbiota could be a potential modulator or a major contributing factor to TME in GI cancer pathogenesis. For example, a high-fat diet triggered carcinogenesis in K-Ras [G12Dint] mice by causing dysbiosis and a decline in Paneth-cell-regulated antimicrobial host defense in the small intestine [56]. These processes negatively affected the recruitment of dendritic cells [DC] and the presentation of MHC class II in the intestine-associated lymphoid tissues [56]. In mouse models of CRC with a knockout *Adenomatous Polyposis Coli* [APC] tumor suppressor gene, impaired intestinal barrier function has been reported at tumor sites. This dysfunction is associated with the proliferation of *Fusobacterium*, leading to additional damage to the mucosal lining [51].

Iida et al. demonstrated that the efficacy of immunotherapy [CpG (cytosine, guanosine, phosphodiester link) oligonucleotides] and oxaliplatin, a platinum-based chemotherapeutic compound, decreased in tumor-bearing mice that lacked microbiota [57]. Several types of tumors were assessed, including colon carcinoma cells. In germ-free mice or those treated with antibiotics, the therapeutic response was significantly diminished in tumor-infiltrating myeloid-derived cells [MDC]. This was accompanied by reduced cytokine synthesis and tumor necrosis following CpG-oligodeoxynucleotide treatment, as well as deficient production of reactive oxygen species [ROS] and cytotoxicity after platinum therapy [57]. The authors concluded that a healthy commensal microbiota, capable of regulating MDC functions, is essential for an effective response to cancer intervention [57].

Compounds produced by intestinal bacteria, such as inosine and lactate, can enhance T cells function and promote anti-tumor immunity. Studies by Mager and co-authors [58] demonstrated that inosine produced by intestinal *B. pseudolongum* caused an exacerbated response to anti-PD-1 and anti-CTL4 treatment. Immunotherapy-induced diminished function of the intestinal barrier was mediated by increased systemic translocation of inosine and led to the activation of antitumor T cells through the increase in expression of adenosine A_{2A} receptor [58]. Another bacterial metabolite, indole-3-lactic acid [ILA] produced by *Lactobacillus plantarum L168,* improved intestinal inflammation, reduced tumor growth through inhibition of Saa3 expression in $CD8^+$ T cells, and alleviated gut dysbiosis in a mouse model of CRC [59]. Notably, ILA stimulated interleukin-12α production in DC, thereby priming CD8+ T cell immunity against neoplastic growth [59]. Moreover, the epigenetic mechanisms of enhancement of tumor-infiltrating $CD8^+$ T cells by ILA were demonstrated [59].

5. BACTERIAL TOXINS AND METABOLITES AFFECTING GI CANCER CELLS

Microbiota-produced enzymes can contribute to adverse effects or resistance to common therapeutics. Studies on colon cancer models have shown that gut bacterial β-glucuronidases can chemically transform Irinotecan [SN38] into its active SN38 form, leading to severe toxic effects, including diarrhea [60]. The enzyme cytidine deaminase, produced intratumorally by *Mycoplasma hyorhinis* and *Escherichia coli*, has been reported to cause resistance to gemcitabine [2',2'-difluorodeoxycytidine] by converting it to its inactive form, 2′,2′-difluorodeoxyuridine, in human PDAC cells and a colon carcinoma mouse model [61]. In a mouse model of multiple intestinal neoplasia, the presence of enterotoxigenic *Bacteroides fragilis* - a human colon resident that produces *B. fragilis* toxin - triggered colitis and strongly induced the formation of colonic tumors via Stat3- and T_H17-mediated pathways [52]. Multiple studies have demonstrated that microbial metabolites, such as short-chain fatty acids [SCFAs], secondary bile acids [BAs], and lipopolysaccharides [LPS], play a key role in GI carcinogenesis [56, 59, 62-64]. The role of the gut microbiota and its metabolites in the pathogenesis and progression of hepatobiliary [HBP] and pancreatic cancers was systematically analyzed by Xu et al. in 2024 [65].

SCFAs are abundantly produced in the colon via the bacterial fermentation of dietary fiber [66, 67]. Particularly, butyric acid, a SCFA acid produced by intratumoral *F. nucleatum*, inhibited HDAC 3/8 in CD8[+] T cells, leading to H3K27 acetylation of the Tbx21 promoter and its expression in humanized mouse models of MSS CRC [32]. Subsequently, TBX21 repressed the transcription of PD-1, reducing CD8[+] T cell exhaustion

and enhancing effector function [32]. Knocking out a butyric acid-producing gene in *F. nucleatum* abrogated its anti-PD1 effect [32]. These data provide insights into the molecular mechanisms by which microbiota affect sensitivity to anti-PD-1 immunotherapies. Butyrate has been shown to reduce the efficacy of CTLA-4 inhibition in a mouse model of colon carcinoma [66]. Furthermore, individuals with advanced CRC have reduced levels of butyrate-producing bacteria and lower concentrations of SCFAs compared to a healthy control group [68-70]. Interestingly, butyrate treatment restored DC recruitment and reduced tumor growth in HFD-fed K-Ras [G12Dint] mouse model of small intestine cancer [56].

Secondary BAs, including deoxycholic acid [DCA] and lithocholic acid [LCA], are produced through the microbial transformation of primary bile acids in the colon [66, 67]. Ou et al. [62] demonstrated that a high-fat and high-protein diet, coupled with low intake of complex carbohydrates, is associated with a higher risk of colon cancer in Americans [62]. It was proposed that the colonic digestive residues resulting from such a diet promote the production of potentially carcinogenic secondary bile acids by microbiota, while reducing the production of antineoplastic SCFAs [62]. The carcinogenic role of DCA was confirmed in a mouse model of an obesity-associated hepatocellular carcinoma [HCC] [71]. LCA, DCA, and other BAs have been shown to activate nuclear receptors FXR and PXR [72], which could lead to changes in the expression of genes responsible for cell proliferation and survival [73] in hepatocytes [63] and colon cancer cells [74, 75]. The role of secondary BAs in GI carcinogenesis remains controversial and requires further studies. For instance, patients with gallbladder cancer [GBC] exhibited significantly decreased levels of DCA, which correlated with poor clinical outcomes [76]. The authors suggested that DCA may play a tumor-suppressive role through miR-92b-3p and PI3K/AKT pathways [76]. Serum levels of LCA were also significantly reduced in GBC patients, and the tumor-suppressive role of LCA, through the inhibition of glutamine metabolic pathways and induction of ferroptosis, was demonstrated in GBC cells and mouse models [77]. Importantly, camptothecin-DCA analogs caused cell cycle arrest at the S and G2/M phases and triggered apoptosis in human liver and colon cancer cells [78]. DCA suppressed the proliferation and migration of pancreatic cancer cells and inhibited epithelial-mesenchymal transition [64]. Similarly, studies in various liver cancer cell lines have demonstrated that functionalized gold nanoparticles LCA inhibit cell proliferation and subsequently activate a ROS-dependent mitochondrial pro-apoptotic pathway [79]. The role of metabolites produced by intestinal microbiota in the pathophysiology of HBP and pancreatic cancers was investigated by Xu et al. in 2024 [65].

Together, these studies highlight the complex effects of bile acids on gastrointestinal carcinogenesis and suggest that targeting BA-induced oncogenic pathways holds therapeutic potential.

6. INDUCTION OF DNA DAMAGE AND MUTAGENESIS DRIVEN BY BACTERIAL GENOTOXINS

Genetic changes leading to the inactivation of tumor suppressor genes, or the activation of oncogenes, are fundamental mechanisms of neoplastic transformation. Pathogenic microorganisms can facilitate these genetic alterations by producing genotoxins, compounds functionally homologous to mammalian type I deoxyribonuclease, which cause breaks in the DNA strands of host cells [80-82]. This process triggers a DNA damage checkpoint pathway and proinflammatory response [83], a common initiator of carcinogenesis and tumorigenesis. Guerra and colleagues investigated the molecular mechanisms by which bacterial genotoxins induce DNA damage and genomic instability [84].

The most well-characterized bacterial genotoxins to date include cytolethal distending toxin [CDT] in gram-negative bacteria, colibactin synthesized by *Escherichia coli [E. coli], and* typhoid toxin produced by *Salmonella enterica serovar Typhi* [85]. Specifically, CDT is synthesized by *Campylobacter spp.* and *E. coli* [86]. These pathogens are found in high abundance in CRC patients [87], particularly in primary CRC lesions with metastasis [88]. Besides, CDT triggered dysplasia in a mouse model of *Helicobacter hepaticus*-induced liver cancer through the pro-inflammatory NF-κB pathway [89]. Exposure to CDT resulted in genetic instability in human cell lines and colorectal organoids derived from biopsy samples of healthy individuals. This instability was attributed to the genotoxic action of the CdtB catalytic subunit, and CDT was identified as a bacterial virulence factor that induces replicative stress and affects proliferating cells, including human colorectal stem cells [90].

Colibactin has been primarily identified in extraintestinal pathogenic *E. coli* strains belonging to the phylogenetic group B2 [91]. This toxin is encoded by the *pks* pathogenicity island [92] and has been shown to alkylate DNA [93], cause DNA double-strand breaks [91], and mutations [94]. Colibactin-producing *E. coli* [CoPEC] was detected at high abundancy in the intestine of CRC patients [95]. In 2010, Cuevas-Ramos and colleagues demonstrated that short-term exposure of cultured mammalian epithelial cells to live pks[+] *E. coli* at low doses triggered a transient DNA damage response [94]. This was followed by cell division showing incomplete DNA repair, resulting in chromosomal abnormalities and the formation of anaphase bridges. The investigators proposed that intestinal colonization with *E. coli* strains harboring the *pks* island might contribute to the development of sporadic CRC [94]. In a similar model, organoids isolated from primary murine colon epithelial cells, briefly infected with *pks+ E. coli*, exhibited characteristics of CRC cells upon recovery, exhibiting a higher proliferation rate and altered differentiation in a *Wnt*-independent manner [92]. In a susceptible mouse model of CRC - $APC^{Min/+}$ mice with a loss-of-function germinal mutation in the *Apc* gene - intestinal tumorigenesis was exacerbated upon colonization by

colibactin-producing *E. coli*. This occurred through the alteration of the autophagic pathway in intestinal epithelial cells [96]. Furthermore, CoPEC modified the tumor microenvironment by triggering an immunosuppressive lipid accumulation, which advanced CRC progression and enhanced chemoresistance [97]. Interestingly, a positive correlation was found between serum IgG antibody titers against the Vi capsular polysaccharide of *Salmonella enterica serovar Typhi* - a component of its typhoid toxin - and gallbladder cancer [GBC] [98]. Previous studies on chronic typhoid and paratyphoid carriers have revealed an increased associated risk for GBC, as well as neoplasms of the pancreas, colorectum, and other organs [99]. Typhoid toxin has been demonstrated to induce hyperphosphorylation of Replication Protein A [RPA], a single-stranded DNA [ssDNA]-binding protein and an indicator of DNA replication stress, in various human cancer cell lines, including colon carcinoma cells [100]. By overwhelming the RPA pathway with an excess of ssDNA substrates, the toxin can cause RPA pool depletion and trigger cellular senescence [100].

In addition to producing genotoxins, microbiota may impact DNA and chromosomal structures through various mechanisms. The abundance of *Fusobacterium nucleatum*, part of the gut microbiota, is associated with specific epigenetic phenotypes and molecular profiles of CRC, including hypermethylation, microsatellite instability [MSI], and mutations in the *BRAF, KRAS, TP53, CHD7*, and *CHD8* genes [101]. FadA, an adhesin protein and a key virulence factor secreted by *F. nucleatum*, has been implicated in the development and progression of CRC through activation of the E-cadherin/β-catenin signaling pathway, which promotes oncogenesis and the expression of inflammatory genes [37]. In $APC^{Min/+}$ mice, FadA has been shown to promote tumorigenesis by inducing DNA damage in colon cancer cells through activation of the E-cadherin/β-catenin pathway and up-regulation of checkpoint kinase 2 [Chk 2] [102]. It has been reported that FadA facilitates CRC progression by inducing the upregulation of long non-coding RNA [lncRNA] LINC00460 and hyperexpression of Annexin A2 [ANXA2] through the competing endogenous RNAs [ceRNAs] network [103]. However, in PDAC, the increased enrichment of *Fusobacterium* did not correlate with any genetic or epigenetic modifications [104]. This suggests that *Fusobacterium* could currently serve only as a prognostic biomarker for pancreatic cancer and not be directly implicated in the pathogenesis of PDAC. Examples of known mechanisms of GI carcinogenesis induced by bacterial toxins are summarized in Table **2**. As shown in human CRC cell lines, another mechanism of DNA damage involves the secretion of EspF, an effector protein produced by enterohemorrhagic *E. coli*, which inhibits the host cell DNA mismatch repair protein [105] and causes oxidative DNA lesions in human intestinal epithelial cells [106]. Chronic inflammation, driven by ROS production from exposure to the toxin of enterotoxigenic *Bacteroides fragilis*, has been implicated as a risk factor for CRC [107]. Similarly, *Enterococcus faecalis*, a bacterium known for producing extracellular superoxide, caused chromosomal and DNA instability in human and murine colon epithelial cell cultures [108].

Table 2. Microbiota-induced DNA damage and its role in GI carcinogenesis.

Bacteria	Genotoxin	Type of Cancer/Model	Molecular and Cellular Mechanisms	Refs.
Campylobacter spp., E. coli	Cytolethal Distending Toxin [CdtB catalytic subunit]	Human colorectal cell lines and organoids	Genetic instability and induction of replicative stress in human colorectal stem cells	[90]
E. coli	Colibactin	Mouse models of CRC	DNA double-strand breaks, mutations, chromosomal aberrations, and promotion of tumorigenesis	[92] [93]
E. coli	Colibactin	Sporadic CRC cell model, human colon cancer cell lines	Transient DNA damage response, incomplete DNA repair, anaphase bridges, chromosome aberrations, aneuploidy, and tetraploidy	[94]
E. coli	Colibactin	Human colon cancer cells, $APC^{Min/+}$ mice	DNA double-strand breaks	[96]
Salmonella enterica serovar Typhi	Typhoid toxin	Colon carcinoma cells	Hyperphosphorylation of RPA, a sensor of single-stranded DNA [ssDNA] and DNA replication stress.	[100]
Fusobacterium spp.	FadA	CRC, $APC^{Min/+}$ mice	Hypermethylation, microsatellite instability [MSI], and mutations in the *BRAF*, *KRAS*, *TP53*, *CHD7*, and *CHD8* genes; DNA damage, Chk2 upregulation, LINC00460 upregulation, and ANXA2 overexpression	[101] [102] [103]

Identifying specific toxin-producing bacteria in patients with GI cancers offers a potential therapeutic strategy by targeting these gut microbiota components. Utilization of small-molecule inhibitors to prevent the biosynthesis of genotoxic and pro-carcinogenic bacterial toxins presents a promising preventive approach.

7. MICROBIOTA TRANSPLANT AS A THERAPEUTIC APPROACH

Currently, metabolomics, a research tool that explores microbial communities and the impact of microbe-derived molecules on the host, has become a crucial aspect of microbiome research [5, 109, 110]Laying the groundwork for the treatment of GI cancers. Moreover, FMT [Fecal Microbiota Transplantation] is a popular tool in preclinical cancer models [19, 50] and holds potential as an effective adjuvant therapy in clinical practice. It is currently being evaluated in several clinical trials for GI cancer Table **3**.

Participants are being recruited for a clinical study to evaluate the safety and efficacy of FMT in minimizing the recurrence of precancerous colorectal adenomas in post-endoscopic resection (NCT06205862), Pancreatic Cancer Cachexia (EXTRA, NCT05606523).

Table 3. Current clinical trials testing fecal microbiota transplantation in GI cancer patients.

Condition	Co-treatment	Clinical Trial Status	Identifier
Colorectal Adenoma	-	Recruiting	NCT06205862
Pancreatic adenocarcinoma / Cachexia	-	Recruiting	NCT05606523
Advanced Gastric Cancer	Chemotherapy with S-1 and Oxaliplatin [SOX], and anti-PD-L1	Recruiting	NCT06346093
Advanced solid tumors originating from the GI tract.	Anti-PD-L1 drugs, including Pembrolizumab or Nivolumab	Completed	NCT05750030
Metastatic Colorectal Cancer in Anti-PD-1 non-responders	Pembrolizumab plus Nivolumab	Phase II	NCT04729322
Unresectable Hepatocellular Carcinoma	Transcatheter Arterial Chemoembolization combined with Lenvatinib plus Sintilimab.	Not yet recruiting	NCT06643533
Advanced Hepatocellular Carcinoma	Atezolizumab and Bevacizumab, Vancomycin	Not yet recruiting	NCT05750030

Given the gut microbiota's ability to influence the effectiveness of cancer immunotherapy or affect drug response, targeted manipulation of gut microbiota could enhance drug efficacy or mitigate adverse effects. Some clinical trials designed for this purpose have been initiated. For instance, the FLORA [Fecal Microbiota Transfer in Liver Cancer to Overcome Resistance to Atezolizumab/Bevacizumab] [NCT05750030] study has been planned to evaluate the safety and immunogenicity of FMT when combined with standard of care immunotherapy. Another study [NCT06346093] is currently recruiting participants to evaluate the efficacy and safety of FMT capsules combined with chemotherapy using S-1 and Oxaliplatin [SOX] and anti-PD-L1 therapy in patients with advanced GC. The findings from the completed clinical trial [NCT05750030] clearly demonstrate that FMT with beneficial microbiota can overcome resistance to anti-PD-1 inhibitors in advanced solid cancers, particularly GI malignancies [30]. Additionally, the effect of FMT combined with the reintroduction of anti-PD-1 antibodies is currently being tested in anti-PD-1 non-responders with metastatic CRC [NCT04729322]. An attempt to reverse drug resistance to the triple therapy regimen with FMT has been planned in a new study involving patients with unresectable hepatocellular carcinoma [NCT06643533]. By testing the efficacy of microbiota-targeted therapies in GI cancer patients, clinical trials can reveal important insights into the role of gut microbiota alterations in shaping cancer treatment outcomes in the future.

CONCLUSION

The reviewed studies collectively underscore the importance of harmonious interactions between the host's digestive system and commensal bacteria [microbiota], which play a pivotal role in maintaining gastrointestinal health. Disruption of this balance, known as gut dysbiosis, triggers a cascade of adverse effects resulting from alterations in the composition, diversity, and functionality of gut microbial communities. Such disruptions can lead to the dominance of particular microbial strains or species that drive carcinogenesis. Mechanisms by which these microorganisms exert their tumorigenic influence include local immunosuppression, weakening the host’s ability to counteract malignant transformation; chronic inflammation, which creates a microenvironment conducive to cancer development; and genotoxicity, which directly damages host DNA, increasing the risk of deleterious mutations.

Furthermore, the ability to profile and characterize gut microbial alterations, coupled with an understanding of their pathophysiological consequences and implicated signaling pathways, offers profound potential in advancing strategies for prevention, early detection, and personalized treatment of GI cancer. This approach highlights the significance of microbiota profiling to guide targeted interventions.

Emerging therapeutic strategies focus on modulating gut microbiota composition to restore balance and reduce cancer-promoting effects. These interventions include supplementation with probiotics and prebiotics to enrich beneficial bacterial populations, precision antibiotic therapies to selectively suppress harmful microbes, and FMT to comprehensively restore a healthy microbial ecosystem. When integrated with conventional treatments, such as chemotherapy and immunotherapy, these microbiota-centered approaches not only improve therapeutic efficacy but may also reduce adverse treatment outcomes, paving the way for more holistic and patient-based cancer management.

AUTHORS’ CONTRIBUTION

It is hereby acknowledged that all authors have accepted responsibility for the manuscript’s content and consented to its submission. They have meticulously reviewed all results and unanimously approved the final version of the manuscript.

LIST OF ABBREVIATIONS

ESCC = Esophageal squamous cell carcinoma

CTLA-4 = Cytotoxic T-lymphocyte-associated protein 4

NSCLC = Non-small-cell lung cancer

ICIs = Immune checkpoint inhibitors

FMT = Fecal microbiota transplantation

GI = Gastrointestinal

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

This study was supported by an intramural grant from Baptist University College of Osteopathic Medicine, Baptist Health Sciences University [2025].

CONFLICT OF INTEREST

The authors declare no conflict of interest financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

REFERENCES

[1] Bray F, Laversanne M, Sung H, *et al*. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2024; 74(3): 229-63. http://dx.doi.org/10.3322/caac.21834 PMID: 38572751

[2] Filho AM, Laversanne M, Ferlay J, Colombet M, Pineros M, Znaor A. The GLOBOCAN 2022 cancer estimates: Data sources, methods, and a snapshot of the cancer burden worldwide. Int J Cancer 2024; 156(7): 1336-46. http://dx.doi.org/10.1002/ijc.35278 PMID: 39688499

[3] Deng Y, Tang D, Hou P, *et al*. Dysbiosis of gut microbiota in patients with esophageal cancer. Microb Pathog 2021; 150: 104709. http://dx.doi.org/10.1016/j.micpath.2020.104709 PMID: 33378710

[4] Ma J, Li J, Jin C, *et al*. Association of gut microbiome and primary liver cancer: A two-sample Mendelian randomization and case–control study. Liver Int 2023; 43(1): 221-33. http://dx.doi.org/10.1111/liv.15466 PMID: 36300678

[5] Kartal E, Schmidt TSB, Molina-Montes E, *et al*. A faecal microbiota signature with high specificity for pancreatic cancer. Gut 2022; 71(7): 1359-72. http://dx.doi.org/10.1136/gutjnl-2021-324755 PMID: 35260444

[6] Sun Y, Zhang X, Jin C, *et al*. Prospective, longitudinal analysis of the gut microbiome in patients with locally advanced rectal cancer predicts response to neoadjuvant concurrent chemoradiotherapy. J Transl Med 2023; 21(1): 221. http://dx.doi.org/10.1186/s12967-023-04054-1 PMID: 36967379

[7] Yachida S, Mizutani S, Shiroma H, *et al*. Metagenomic and metabolomic analyses reveal distinct stage-specific phenotypes of the gut microbiota in colorectal cancer. Nat Med 2019; 25(6): 968-76. http://dx.doi.org/10.1038/s41591-019-0458-7 PMID: 31171880

[8] Scott AJ, Alexander JL, Merrifield CA, *et al*. International Cancer Microbiome Consortium consensus statement on the role of the human microbiome in carcinogenesis. Gut 2019; 68(9): 1624-32. http://dx.doi.org/10.1136/gutjnl-2019-318556 PMID: 31092590

[9] Tumeh PC, Harview CL, Yearley JH, *et al*. PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature 2014; 515(7528): 568-71. http://dx.doi.org/10.1038/nature13954 PMID: 25428505

[10] Gopalakrishnan V, Spencer CN, Nezi L, *et al*. Gut microbiome modulates response to anti–PD-1 immunotherapy in melanoma patients. Science 2018; 359(6371): 97-103. http://dx.doi.org/10.1126/science.aan4236 PMID: 29097493

[11] Garon EB, Rizvi NA, Hui R, *et al*. Pembrolizumab for the treatment of non-small-cell lung cancer. N Engl J Med 2015; 372(21): 2018-28. http://dx.doi.org/10.1056/NEJMoa1501824 PMID: 25891174

[12] Overman MJ, McDermott R, Leach JL, *et al*. Nivolumab in patients with metastatic DNA mismatch repair-deficient or microsatellite instability-high colorectal cancer (CheckMate 142): An open-label, multicentre, phase 2 study. Lancet Oncol 2017; 18(9): 1182-91. http://dx.doi.org/10.1016/S1470-2045(17)30422-9 PMID: 28734759

[13] Gunjur A, Shao Y, Rozday T, *et al*. A gut microbial signature for combination immune checkpoint blockade across cancer types. Nat Med 2024; 30(3): 797-809. http://dx.doi.org/10.1038/s41591-024-02823-z PMID: 38429524

[14] Comprehensive molecular characterization of gastric adenocarcinoma. Nature 2014; 513(7517): 202-9. http://dx.doi.org/10.1038/nature13480 PMID: 25079317

[15] Tanaka K, Miyata H, Sugimura K, *et al*. Negative influence of programmed death-1-ligands on the survival of esophageal cancer patients treated with chemotherapy. Cancer Sci 2016; 107(6): 726-33. http://dx.doi.org/10.1111/cas.12938 PMID: 27015293

[16] Zheng Z, Bu Z, Liu X, *et al*. Level of circulating PD-L1 expression in patients with advanced gastric cancer and its clinical implications. Chin J Cancer Res 2014; 26(1): 104-11. http://dx.doi.org/10.3978/j.issn.1000-9604.2014.02.08 PMID: 24653632

[17] Leach DR, Krummel MF, Allison JP. Enhancement of antitumor immunity by CTLA-4 blockade. Science 1996; 271(5256): 1734-6. http://dx.doi.org/10.1126/science.271.5256.1734 PMID: 8596936

[18] Waterhouse P, Penninger JM, Timms E, *et al*. Lymphoproliferative disorders with early lethality in mice deficient in Ctla-4. Science 1995; 270(5238): 985-8. http://dx.doi.org/10.1126/science.270.5238.985 PMID: 7481803

[19] Routy B, Le Chatelier E, Derosa L, *et al*. Gut microbiome influences efficacy of PD-1–based immunotherapy against epithelial tumors. Science 2018; 359(6371): 91-7. http://dx.doi.org/10.1126/science.aan3706 PMID: 29097494

[20] Sivan A, Corrales L, Hubert N, *et al*. Commensal *Bifidobacterium* promotes antitumor immunity and facilitates anti–PD-L1 efficacy. Science 2015; 350(6264): 1084-9. http://dx.doi.org/10.1126/science.aac4255 PMID: 26541606

[21] Davar D, Dzutsev AK, McCulloch JA, *et al*. Fecal microbiota transplant overcomes resistance to anti–PD-1 therapy in melanoma patients. Science 2021; 371(6529): 595-602. http://dx.doi.org/10.1126/science.abf3363 PMID: 33542131

[22] Matson V, Fessler J, Bao R, *et al*. The commensal microbiome is associated with anti–PD-1 efficacy in metastatic melanoma patients. Science 2018; 359(6371): 104-8. http://dx.doi.org/10.1126/science.aao3290 PMID: 29302014

[23] Janjigian YY, Ajani JA, Moehler M, *et al*. First-line nivolumab plus chemotherapy for advanced gastric, gastroesophageal junction, and esophageal adenocarcinoma: 3-year follow-up of the phase III checkmate 649 trial. J Clin Oncol 2024; 42(17): 2012-20. http://dx.doi.org/10.1200/JCO.23.01601 PMID: 38382001

[24] Sorscher S, Resnick J, Goodman M. First case report of a dramatic radiographic response to a checkpoint inhibitor in a patient with proficient mismatch repair gene expressing metastatic colorectal cancer. JCO Precis Oncol 2017; 1(1): 1-4. http://dx.doi.org/10.1200/PO.16.00005 PMID: 35172480

[25] Kawazoe A, Kuwata T, Kuboki Y, *et al*. Clinicopathological features of programmed death ligand 1 expression with tumor-infiltrating lymphocyte, mismatch repair, and Epstein–Barr virus status in a large cohort of gastric cancer patients. Gastric Cancer 2017; 20(3): 407-15. http://dx.doi.org/10.1007/s10120-016-0631-3 PMID: 27629881

[26] Koganemaru S, Inoshita N, Miura Y, *et al*. Prognostic value of programmed death-ligand 1 expression in patients with stage III colorectal cancer. Cancer Sci 2017; 108(5): 853-8. http://dx.doi.org/10.1111/cas.13229 PMID: 28267224

[27] Kim R, Keam B, Kwon D, *et al*. Programmed death ligand-1 expression and its prognostic role in esophageal squamous cell carcinoma. World J Gastroenterol 2016; 22(37): 8389-97. http://dx.doi.org/10.3748/wjg.v22.i37.8389 PMID: 27729745

[28] Yap DWT, Leone AG, Wong NZH, *et al*. Effectiveness of immune checkpoint inhibitors in patients with advanced esophageal squamous cell carcinoma. JAMA Oncol 2023; 9(2): 215-24. http://dx.doi.org/10.1001/jamaoncol.2022.5816 PMID: 36480211

[29] Wu Z, Zhang S, Li L, Huang Z, Huang D, Hu Y. The gut microbiota modulates responses to anti–PD-1 and chemotherapy combination therapy and related adverse events in patients with advanced solid tumors. Front Oncol 2022; 12: 887383. http://dx.doi.org/10.3389/fonc.2022.887383 PMID: 36387171

[30] Kim Y, Kim G, Kim S, *et al*. Fecal microbiota transplantation improves anti-PD-1 inhibitor efficacy in unresectable or metastatic solid cancers refractory to anti-PD-1 inhibitor. Cell Host Microbe 2024; 32(8): 1380-1393.e9. http://dx.doi.org/10.1016/j.chom.2024.06.010 PMID: 39059396

[31] Peng Z, Cheng S, Kou Y, *et al*. The gut microbiome is associated with clinical response to anti–PD-1/PD-L1 immunotherapy in gastrointestinal cancer. Cancer Immunol Res 2020; 8(10): 1251-61. http://dx.doi.org/10.1158/2326-6066.CIR-19-1014 PMID: 32855157

[32] Wang X, Fang Y, Liang W, *et al*. Fusobacterium nucleatum facilitates anti-PD-1 therapy in microsatellite stable colorectal cancer. Cancer Cell 2024; 42(10): 1729-1746.e8. http://dx.doi.org/10.1016/j.ccell.2024.08.019 PMID: 39303724

[33] Mao J, Wang D, Long J, *et al*. Gut microbiome is associated with the clinical response to anti-PD-1 based immunotherapy in hepatobiliary cancers. J Immunother Cancer 2021; 9(12): 003334. http://dx.doi.org/10.1136/jitc-2021-003334 PMID: 34873013

[34] Mima K, Nishihara R, Qian ZR, *et al*. *Fusobacterium nucleatum* in colorectal carcinoma tissue and patient prognosis. Gut 2016; 65(12): 1973-80. http://dx.doi.org/10.1136/gutjnl-2015-310101 PMID: 26311717

[35] Zhou Z, Chen J, Yao H, Hu H. *Fusobacterium* and colorectal cancer. Front Oncol 2018; 8: 371. http://dx.doi.org/10.3389/fonc.2018.00371 PMID: 30374420

[36] Kostic AD, Chun E, Robertson L, *et al*. Fusobacterium nucleatum potentiates intestinal tumorigenesis and modulates the tumor-immune microenvironment. Cell Host Microbe 2013; 14(2): 207-15. http://dx.doi.org/10.1016/j.chom.2013.07.007 PMID: 23954159

[37] Rubinstein MR, Wang X, Liu W, Hao Y, Cai G, Han YW. Fusobacterium nucleatum promotes colorectal carcinogenesis by modulating E-cadherin/β-catenin signaling *via* its FadA adhesin. Cell Host Microbe 2013; 14(2): 195-206. http://dx.doi.org/10.1016/j.chom.2013.07.012 PMID: 23954158

[38] Galeano Niño JL, Wu H, LaCourse KD, *et al*. Effect of the intratumoral microbiota on spatial and cellular heterogeneity in cancer. Nature 2022; 611(7937): 810-7. http://dx.doi.org/10.1038/s41586-022-05435-0 PMID: 36385528

[39] Zepeda-Rivera M, Minot SS, Bouzek H, *et al*. A distinct Fusobacterium nucleatum clade dominates the colorectal cancer niche. Nature 2024; 628(8007): 424-32. http://dx.doi.org/10.1038/s41586-024-07182-w PMID: 38509359

[40] Bullman S, Pedamallu CS, Sicinska E, *et al*. Analysis of *Fusobacterium* persistence and antibiotic response in colorectal cancer. Science 2017; 358(6369): 1443-8. http://dx.doi.org/10.1126/science.aal5240 PMID: 29170280

[41] LaCourse KD, Zepeda-Rivera M, Kempchinsky AG, *et al*. The cancer chemotherapeutic 5-fluorouracil is a potent Fusobacterium nucleatum inhibitor and its activity is modified by intratumoral microbiota. Cell Rep 2022; 41(7): 111625. http://dx.doi.org/10.1016/j.celrep.2022.111625 PMID: 36384132

[42] Marshall BJ, Armstrong JA, McGechie DB, Clancy RJ. Attempt to fulfil Koch's postulates for pyloric Campylobacter. Med J Aust 1985; 142(8): 436-9. http://dx.doi.org/10.5694/j.1326-5377.1985.tb113443.x PMID: 3982345

[43] Marshall B, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet 1984; 323(8390): 1311-5. http://dx.doi.org/10.1016/S0140-6736(84)91816-6 PMID: 6145023

[44] Lehouritis P, Cummins J, Stanton M, *et al*. Local bacteria affect the efficacy of chemotherapeutic drugs. Sci Rep 2015; 5(1): 14554. http://dx.doi.org/10.1038/srep14554 PMID: 26416623

[45] Mohindroo C, Hasanov M, Rogers JE, *et al*. Antibiotic use influences outcomes in advanced pancreatic adenocarcinoma patients. Cancer Med 2021; 10(15): 5041-50. http://dx.doi.org/10.1002/cam4.3870 PMID: 34250759

[46] Zaidi AH, Kelly LA, Kreft RE, *et al*. Associations of microbiota and toll-like receptor signaling pathway in esophageal adenocarcinoma. BMC Cancer 2016; 16(1): 52. http://dx.doi.org/10.1186/s12885-016-2093-8 PMID: 26841926

[47] Sawada A, Fujiwara Y, Nagami Y, *et al*. Alteration of esophageal microbiome by antibiotic treatment does not affect incidence of rat esophageal adenocarcinoma. Dig Dis Sci 2016; 61(11): 3161-8. http://dx.doi.org/10.1007/s10620-016-4263-6 PMID: 27461059

[48] Lertpiriyapong K, Whary MT, Muthupalani S, *et al*. Gastric colonisation with a restricted commensal microbiota replicates the promotion of neoplastic lesions by diverse intestinal microbiota in the *Helicobacter pylori* INS-GAS mouse model of gastric carcinogenesis. Gut 2014; 63(1): 54-63. http://dx.doi.org/10.1136/gutjnl-2013-305178 PMID: 23812323

[49] Arita S, Inagaki-Ohara K. High-fat-diet–induced modulations of leptin signaling and gastric microbiota drive precancerous lesions in the stomach. Nutrition 2019; 67-68: 110556. http://dx.doi.org/10.1016/j.nut.2019.110556 PMID: 31554603

[50] Chen X, Zhou B, Wang S, *et al*. Intestinal metaplasia key molecules and UPP1 activation *via* Helicobacter pylori /NF-kB: Drivers of malignant progression in gastric cancer. Cancer Cell Int 2024; 24(1): 399. http://dx.doi.org/10.1186/s12935-024-03598-6 PMID: 39695769

[51] Grivennikov SI, Wang K, Mucida D, *et al*. Adenoma-linked barrier defects and microbial products drive IL-23/IL-17-mediated tumour growth. Nature 2012; 491(7423): 254-8. http://dx.doi.org/10.1038/nature11465 PMID: 23034650

[52] Wu S, Rhee KJ, Albesiano E, *et al*. A human colonic commensal promotes colon tumorigenesis *via* activation of T helper type 17 T cell responses. Nat Med 2009; 15(9): 1016-22. http://dx.doi.org/10.1038/nm.2015 PMID: 19701202

[53] Galon J, Costes A, Sanchez-Cabo F, *et al*. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science 2006; 313(5795): 1960-4. http://dx.doi.org/10.1126/science.1129139 PMID: 17008531

[54] Atarashi K, Tanoue T, Shima T, *et al*. Induction of colonic regulatory T cells by indigenous Clostridium species. Science 2011; 331(6015): 337-41. http://dx.doi.org/10.1126/science.1198469 PMID: 21205640

[55] Ivanov II, Atarashi K, Manel N, *et al*. Induction of intestinal Th17 cells by segmented filamentous bacteria. Cell 2009; 139(3): 485-98. http://dx.doi.org/10.1016/j.cell.2009.09.033 PMID: 19836068

[56] Schulz MD, Atay Ç, Heringer J, *et al*. High-fat-diet-mediated dysbiosis promotes intestinal carcinogenesis independently of obesity. Nature 2014; 514(7523): 508-12. http://dx.doi.org/10.1038/nature13398 PMID: 25174708

[57] Iida N, Dzutsev A, Stewart CA, *et al*. Commensal bacteria control cancer response to therapy by modulating the tumor microenvironment. Science 2013; 342(6161): 967-70. http://dx.doi.org/10.1126/science.1240527 PMID: 24264989

[58] Mager LF, Burkhard R, Pett N, *et al*. Microbiome-derived inosine modulates response to checkpoint inhibitor immunotherapy. Science 2020; 369(6510): 1481-9. http://dx.doi.org/10.1126/science.abc3421 PMID: 32792462

[59] Zhang Q, Zhao Q, Li T, *et al*. Lactobacillus plantarum-derived indole-3-lactic acid ameliorates colorectal tumorigenesis *via* epigenetic regulation of $CD8^+$ T cell immunity. Cell Metab 2023; 35(6): 943-960.e9. http://dx.doi.org/10.1016/j.cmet.2023.04.015 PMID: 37192617

[60] Wallace BD, Wang H, Lane KT, *et al*. Alleviating cancer drug toxicity by inhibiting a bacterial enzyme. Science 2010; 330(6005): 831-5. http://dx.doi.org/10.1126/science.1191175 PMID: 21051639

[61] Geller LT, Barzily-Rokni M, Danino T, *et al*. Potential role of intratumor bacteria in mediating tumor resistance to the chemotherapeutic drug gemcitabine. Science 2017; 357(6356): 1156-60. http://dx.doi.org/10.1126/science.aah5043 PMID: 28912244

[62] Ou J, DeLany JP, Zhang M, Sharma S, O'Keefe SJD. Association between low colonic short-chain fatty acids and high bile acids in high colon cancer risk populations. Nutr Cancer 2012; 64(1): 34-40. http://dx.doi.org/10.1080/01635581.2012.630164 PMID: 22136517

[63] Xie G, Wang X, Huang F, *et al*. Dysregulated hepatic bile acids collaboratively promote liver carcinogenesis. Int J Cancer 2016; 139(8): 1764-75. http://dx.doi.org/10.1002/ijc.30219 PMID: 27273788

[64] Zhu S, Yang K, Yang S, *et al*. A high bile acid environment promotes apoptosis and inhibits migration in pancreatic cancer. Bioengineered 2022; 13(3): 6719-28. http://dx.doi.org/10.1080/21655979.2022.2045823 PMID: 35245979

[65] Xu Y, Le J, Qin J, *et al*. Decoding the microbiota metabolome in hepatobiliary and pancreatic cancers: Pathways to precision diagnostics and targeted therapeutics. Pharmacol Res 2024; 208: 107364. http://dx.doi.org/10.1016/j.phrs.2024.107364 PMID: 39181345

[66] Coutzac C, Jouniaux JM, Paci A, *et al*. Systemic short chain fatty acids limit antitumor effect of CTLA-4 blockade in hosts with cancer. Nat Commun 2020; 11(1): 2168. http://dx.doi.org/10.1038/s41467-020-16079-x PMID: 32358520

[67] Cummings JH, Pomare EW, Branch WJ, Naylor CP, Macfarlane GT. Short chain fatty acids in human large intestine, portal, hepatic and venous blood. Gut 1987; 28(10): 1221-7. http://dx.doi.org/10.1136/gut.28.10.1221 PMID: 3678950

[68] Chen HM, Yu YN, Wang JL, *et al*. Decreased dietary fiber intake and structural alteration of gut microbiota in patients with advanced colorectal adenoma. Am J Clin Nutr 2013; 97(5): 1044-52. http://dx.doi.org/10.3945/ajcn.112.046607 PMID: 23553152

[69] Hu S, Liu L, Chang EB, Wang JY, Raufman JP. Butyrate inhibits pro-proliferative miR-92a by diminishing c-Myc-induced miR-17-92a cluster transcription in human colon cancer cells. Mol Cancer 2015; 14(1): 180. http://dx.doi.org/10.1186/s12943-015-0450-x PMID: 26463716

[70] Humphreys KJ, Conlon MA, Young GP, *et al*. Dietary manipulation of oncogenic microRNA expression in human rectal mucosa: A randomized trial. Cancer Prev Res 2014; 7(8): 786-95. http://dx.doi.org/10.1158/1940-6207.CAPR-14-0053 PMID: 25092886

[71] Yoshimoto S, Loo TM, Atarashi K, *et al*. Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. Nature 2013; 499(7456): 97-101. http://dx.doi.org/10.1038/nature12347 PMID: 23803760

[72] Makishima M, Okamoto AY, Repa JJ, *et al*. Identification of a nuclear receptor for bile acids. Science 1999; 284(5418): 1362-5. http://dx.doi.org/10.1126/science.284.5418.1362 PMID: 10334992

[73] Kong B, Wang L, Chiang JYL, Zhang Y, Klaassen CD, Guo GL. Mechanism of tissue-specific farnesoid X receptor in suppressing the expression of genes in bile-acid synthesis in mice. Hepatology 2012; 56(3): 1034-43. http://dx.doi.org/10.1002/hep.25740 PMID: 22467244

[74] Cheng K, Raufman JP. Bile acid-induced proliferation of a human colon cancer cell line is mediated by transactivation of epidermal growth factor receptors. Biochem Pharmacol 2005; 70(7): 1035-47. http://dx.doi.org/10.1016/j.bcp.2005.07.023 PMID: 16139803

[75] Ha YH, Park DG. Effects of DCA on cell cycle proteins in colonocytes. J Korean Soc Coloproctol 2010; 26(4): 254-9. http://dx.doi.org/10.3393/jksc.2010.26.4.254 PMID: 21152226

[76] Lin R, Zhan M, Yang L, *et al*. Deoxycholic acid modulates the progression of gallbladder cancer through N^6-methyladenosine-dependent microRNA maturation. Oncogene 2020; 39(26): 4983-5000. http://dx.doi.org/10.1038/s41388-020-1349-6 PMID: 32514152

[77] Li W, Wang Z, Lin R, *et al*. Lithocholic acid inhibits gallbladder cancer proliferation through interfering glutaminase-mediated glutamine metabolism. Biochem Pharmacol 2022; 205: 115253. http://dx.doi.org/10.1016/j.bcp.2022.115253 PMID: 36176239

[78] Xiao L, Xu J, Weng Q, *et al*. Mechanism of a novel camptothecin-deoxycholic acid derivate induced apoptosis against human liver cancer HepG2 cells and human colon cancer HCT116 cells. Recent Patents Anticancer Drug Discov 2020; 14(4): 370-82. http://dx.doi.org/10.2174/1574892814666191016162346 PMID: 31644410

[79] Zhao MX, Cai ZC, Zhu BJ, Zhang ZQ. The apoptosis effect on liver cancer cells of gold nanoparticles modified with lithocholic acid. Nanoscale Res Lett 2018; 13(1): 304. http://dx.doi.org/10.1186/s11671-018-2653-8 PMID: 30269179

[80] Elwell CA, Dreyfus LA. DNase I homologous residues in CdtB are critical for cytolethal distending toxin-mediated cell cycle arrest. Mol Microbiol 2000; 37(4): 952-63. http://dx.doi.org/10.1046/j.1365-2958.2000.02070.x PMID: 10972814

[81] Nešić D, Hsu Y, Stebbins CE. Assembly and function of a bacterial genotoxin. Nature 2004; 429(6990): 429-33. http://dx.doi.org/10.1038/nature02532 PMID: 15164065

[82] Toller IM, Neelsen KJ, Steger M, *et al*. Carcinogenic bacterial pathogen *Helicobacter pylori* triggers DNA double-strand breaks and a DNA damage response in its host cells. Proc Natl Acad Sci USA 2011; 108(36): 14944-9. http://dx.doi.org/10.1073/pnas.1100959108 PMID: 21896770

[83] Hassane DC, Lee RB, Pickett CL. Campylobacter jejuni cytolethal distending toxin promotes DNA repair responses in normal human cells. Infect Immun 2003; 71(1): 541-5. http://dx.doi.org/10.1128/IAI.71.1.541-545.2003 PMID: 12496208

[84] Guerra L, Guidi R, Frisan T. Do bacterial genotoxins contribute to chronic inflammation, genomic instability and tumor progression? FEBS J 2011; 278(23): 4577-88. http://dx.doi.org/10.1111/j.1742-4658.2011.08125.x PMID: 21585655

[85] Grasso F, Frisan T. Bacterial genotoxins: Merging the DNA damage response into infection biology. Biomolecules 2015; 5(3): 1762-82. http://dx.doi.org/10.3390/biom5031762 PMID: 26270677

[86] Johnson WM, Lior H. A new heat-labile cytolethal distending toxin (CLDT) produced by Campylobacter spp. Microb Pathog 1988; 4(2): 115-26. http://dx.doi.org/10.1016/0882-4010(88)90053-8 PMID: 2849028

[87] Warren RL, Freeman DJ, Pleasance S, *et al*. Co-occurrence of anaerobic bacteria in colorectal carcinomas. Microbiome 2013; 1(1): 16. http://dx.doi.org/10.1186/2049-2618-1-16 PMID: 24450771

[88] He Z, Yu J, Gong J, *et al*. Campylobacter jejuni-derived cytolethal distending toxin promotes colorectal cancer metastasis. Cell Host Microbe 2024; 32(12): 2080-2091.e6. http://dx.doi.org/10.1016/j.chom.2024.11.006 PMID: 39626677

[89] Ge Z, Rogers AB, Feng Y, *et al*. Bacterial cytolethal distending toxin promotes the development of dysplasia in a model of microbially induced hepatocarcinogenesis. Cell Microbiol 2007; 9(8): 2070-80. http://dx.doi.org/10.1111/j.1462-5822.2007.00939.x PMID: 17441986

[90] Tremblay W, Mompart F, Lopez E, *et al*. Cytolethal distending toxin promotes replicative stress leading to genetic instability transmitted to daughter cells. Front Cell Dev Biol 2021; 9: 656795.

http://dx.doi.org/10.3389/fcell.2021.656795 PMID: 34026755

[91] Nougayrède JP, Homburg S, Taieb F, *et al*. Escherichia coli induces DNA double-strand breaks in eukaryotic cells. Science 2006; 313(5788): 848-51. http://dx.doi.org/10.1126/science.1127059 PMID: 16902142

[92] Iftekhar A, Berger H, Bouznad N, *et al*. Genomic aberrations after short-term exposure to colibactin-producing E. coli transform primary colon epithelial cells. Nat Commun 2021; 12(1): 1003. http://dx.doi.org/10.1038/s41467-021-21162-y PMID: 33579932

[93] Wilson MR, Jiang Y, Villalta PW, *et al*. The human gut bacterial genotoxin colibactin alkylates DNA. Science 2019; 363(6428): eaar7785. http://dx.doi.org/10.1126/science.aar7785 PMID: 30765538

[94] Cuevas-Ramos G, Petit CR, Marcq I, Boury M, Oswald E, Nougayrède JP. *Escherichia coli* induces DNA damage *in vivo* and triggers genomic instability in mammalian cells. Proc Natl Acad Sci USA 2010; 107(25): 11537-42. http://dx.doi.org/10.1073/pnas.1001261107 PMID: 20534522

[95] Moore WE, Moore LH. Intestinal floras of populations that have a high risk of colon cancer. Appl Environ Microbiol 1995; 61(9): 3202-7. http://dx.doi.org/10.1128/aem.61.9.3202-3207.1995 PMID: 7574628

[96] Lucas C, Salesse L, Hoang MHT, *et al*. Autophagy of intestinal epithelial cells inhibits colorectal carcinogenesis induced by colibactin-producing escherichia coli in apc mice. Gastroenterology 2020; 158(5): 1373-88. http://dx.doi.org/10.1053/j.gastro.2019.12.026 PMID: 31917256

[97] de Oliveira Alves N, Dalmasso G, Nikitina D, *et al*. The colibactin-producing *Escherichia coli* alters the tumor microenvironment to immunosuppressive lipid overload facilitating colorectal cancer progression and chemoresistance. Gut Microbes 2024; 16(1): 2320291. http://dx.doi.org/10.1080/19490976.2024.2320291 PMID: 38417029

[98] Koshiol J, Wozniak A, Cook P, *et al*. *Salmonella enterica* serovar Typhi and gallbladder cancer: A case–control study and meta-analysis. Cancer Med 2016; 5(11): 3310-235. http://dx.doi.org/10.1002/cam4.915 PMID: 27726295

[99] Caygill CPJ, Hill MJ, Braddick M, Sharp JCM. Cancer mortality in chronic typhoid and paratyphoid carriers. Lancet 1994; 343(8889): 83-4. http://dx.doi.org/10.1016/S0140-6736(94)90816-8 PMID: 7903779

[100] Ibler AEM, ElGhazaly M, Naylor KL, Bulgakova NAF, F El-Khamisy S, Humphreys D. Typhoid toxin exhausts the RPA response to DNA replication stress driving senescence and Salmonella infection. Nat Commun 2019; 10(1): 4040. http://dx.doi.org/10.1038/s41467-019-12064-1 PMID: 31492859

[101] Tahara T, Yamamoto E, Suzuki H, *et al*. Fusobacterium in colonic flora and molecular features of colorectal carcinoma. Cancer Res 2014; 74(5): 1311-8. http://dx.doi.org/10.1158/0008-5472.CAN-13-1865 PMID: 24385213

[102] Guo P, Tian Z, Kong X, *et al*. FadA promotes DNA damage and progression of Fusobacterium nucleatum-induced colorectal cancer through up-regulation of chk2. J Exp Clin Cancer Res 2020; 39(1): 202. http://dx.doi.org/10.1186/s13046-020-01677-w PMID: 32993749

[103] Rezasoltani S, Shams E, Piroozkhah M, *et al*. FadA antigen of Fusobacterium nucleatum: Implications for ceRNA network in colorectal cancer and adenomatous polyps progression. Discov Oncol 2025; 16(1): 58. http://dx.doi.org/10.1007/s12672-025-01796-w PMID: 39826054

[104] Mitsuhashi K, Nosho K, Sukawa Y, *et al*. Association of *Fusobacterium* species in pancreatic cancer tissues with molecular features and prognosis. Oncotarget 2015; 6(9): 7209-20. http://dx.doi.org/10.18632/oncotarget.3109 PMID: 25797243

[105] Maddocks ODK, Scanlon KM, Donnenberg MS. An Escherichia coli effector protein promotes host mutation *via* depletion of DNA mismatch repair proteins. MBio 2013; 4(3): e00152-13. http://dx.doi.org/10.1128/mBio.00152-13 PMID: 23781066

[106] Fang Y, Fu M, Li X, Zhang B, Wan C. Enterohemorrhagic *Escherichia coli* effector EspF triggers oxidative DNA lesions in intestinal epithelial cells. Infect Immun 2024; 92(4): e00001-24. http://dx.doi.org/10.1128/iai.00001-24 PMID: 38415639

[107] Goodwin AC, Shields CED, Wu S, *et al*. Polyamine catabolism contributes to enterotoxigenic *Bacteroides fragilis* -Induced colon tumorigenesis. Proc Natl Acad Sci USA 2011; 108(37): 15354-9. http://dx.doi.org/10.1073/pnas.1010203108 PMID: 21876161

[108] Wang X, Allen TD, May RJ, Lightfoot S, Houchen CW, Huycke MM. Enterococcus faecalis induces aneuploidy and tetraploidy in colonic epithelial cells through a bystander effect. Cancer Res 2008; 68(23): 9909-17. http://dx.doi.org/10.1158/0008-5472.CAN-08-1551 PMID: 19047172

[109] Han S, Van Treuren W, Fischer CR, *et al*. A metabolomics pipeline for the mechanistic interrogation of the gut microbiome. Nature 2021; 595(7867): 415-20. http://dx.doi.org/10.1038/s41586-021-03707-9 PMID: 34262212

[110] Thompson LR, Sanders JG, McDonald D, *et al*. A communal catalogue reveals Earth's multiscale microbial diversity. Nature 2017; 551(7681): 457-63. http://dx.doi.org/10.1038/nature24621 PMID: 29088705

DISCLAIMER: The above article has been published, as is, ahead-of-print, to provide early visibility but is not the final version. Major publication processes like copyediting, proofing, typesetting and further review are still to be done and may lead to changes in the final published version, if it is eventually published. All legal disclaimers that apply to the final published article also apply to this ahead-of-print version.