Chinese Medical Journal®

Clostridioides difficile: A suspected pro-carcinogenic bacterium for gastrointestinal tumors

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Abstract

Gastrointestinal tumors are among the most prevalent and deadly cancers worldwide and have been increasingly associated with the gut microbiota. Particularly, colorectal cancer (CRC) has become a focal point for unraveling the complex interplay between microbial dynamics and gastrointestinal tumor development, as extensive studies have shown that gut microbiota dysbiosis is closely associated with CRC, affecting energy harvest, metabolism, and mucosal and systemic immune responses. *Clostridioides difficile* (*C. difficile*) is the major causative agent of gut microbiota dysbiosis, with toxins A and B being its main pathogenic factors. These toxins reportedly trigger a complex cascade of host cellular responses, leading to diarrhea, inflammation, and tissue necrosis. However, recent experimental evidence suggests that chronic infection with *C. difficile* is a previously unrecognized contributor to colonic tumorigenesis. In this concise review, we summarize the hypothetical models and provide a comprehensive overview of the mechanisms linking the microbiota to colorectal carcinogenesis, focusing on the reasonable extrapolation of the interaction between *C. difficile* and CRC. Understanding the significance of *C. difficile* as a potential pro-carcinogenic bacterium and its potential role as a biomarker in CRC is crucial for advancing our knowledge in preventing tumorigenesis, recurrence, and gastrointestinal tumor metastasis.

Keywords: *Clostridioides difficile*; Colorectal cancer; Enteric microorganism; Gastrointestinal tumors; Mechanism

Introduction

The gut microbiota is a complex community of ~100 trillion microorganisms, including bacteria, viruses, fungi, archaea, and eukaryotic organisms,[1] playing a role in the host's physiology, including metabolic and signal transduction, nutrition, immune development, and host defense.[2] Several intrinsic and environmental factors influence the gut microbial community in healthy individuals, including age,[3] diet,[4] drugs,[5] sports,[6] smoking,[7] host genotype,[8] and sex hormones.[9] Alterations in the gut microbiota composition by these host intrinsic and extrinsic factors result in dysbiosis, which is associated with various diseases, including inflammatory bowel disease (IBD),[10] irritable bowel syndrome,[11] type 2 diabetes,[12] obesity,[13] liver disease,[14] cardiometabolic disease,[15] skin diseases,[16] and particularly gastrointestinal tumors.[17,18]

Gastrointestinal malignancies, including colorectal cancer (CRC), gastric cancer, pancreatic cancer, esophageal cancer, hepatocellular carcinoma, and gallbladder and biliary tract cancers, constitute a major global health burden. Collectively, these tumors account for more than one-quarter of all cancer diagnoses and over one-third of cancer-related mortality worldwide, underscoring the urgent need for improved strategies for prevention, diagnosis, and treatment.[19] Among these malignancies, CRC stands out due to its high incidence and mortality rates. In 2022, CRC was the third most diagnosed cancer worldwide, accounting for 9.6% of all new cancer cases, and the second leading cause of cancer-related death (9.3% of all cancer-related deaths).[20] Due to the substantial burden it imposes,[22] CRC has garnered attention as a representative model for studying microbiota-associated mechanisms of tumorigenesis in the digestive tract.

The first correlation of gut microbiota with CRC was reported in 1975, following a 20% CRC development in germ-free rats compared with 93% in conventional rats.[21] Afterward, the "oncomicrobiotic" relationship

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.1097/CM9.0000000000003834

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Chinese Medical Journal 2026;139(1)

Received: 03-12-2024; **Online:** 20-11-2025 **Edited by:** Yanjie Yin

was extensively explored in CRC due to the natural proximity of the commensal bacteria to the colonic mucosa.[17] Although multiple gut bacteria reportedly drive the oncogenic process, no bacterium has been confirmed as a direct cause of CRC. Experimental evidence suggests that bacteria may contribute to tumorigenesis through diverse pathways, including DNA damage caused by bacterial toxins; pro-oncogenic metabolites, especially those linked to dietary patterns; chronic inflammation arising from host–microbe interactions and biofilm formation; and impairment of host anti-tumor immunity.[22] This suggests that factors, such as host lifestyle, microbiome interactions, and immune responses—mucosal and systemic—may be critical in this process.

Although it has been traditionally studied in the context of antibiotic-associated diarrhea and healthcare-associated infections, research on the role of *Clostridioides difficile* (*C. difficile*) in gastrointestinal tract tumors is limited. As *C. difficile* infection (CDI) primarily involves the colon, especially the distal colon and rectum, research on its association with gastrointestinal tumors has largely focused on its potential link to CRC. Studies have observed a higher prevalence of CDI in CRC patients,[23–26] raising the question of whether this is a coincidental consequence of colonic disease and immunosuppression or whether *C. difficile* plays an active role in promoting carcinogenesis. *C. difficile*, a notorious pathogen known for its toxin production, gut microbiota disruption, and host immunity modulation, may contribute to colorectal carcinogenesis through mechanisms similar to those identified in previously well-characterized oncomicrobes.[22] Several plausible tumorigenic effects could link CDI to CRC, including (1) direct effects, such as DNA damage caused by *C. difficile* toxins, which can induce mutations in host intestinal epithelial cells (IECs) and (2) indirect effects, involving chronic inflammation, immune dysregulation, microbial dysbiosis, or the production of pro-carcinogenic metabolites triggered by *C. difficile* toxins or other pathogenic factors. Consequently, the potential interactions between *C. difficile* and other microbial or host-related risk factors may synergistically contribute to colorectal tumorigenesis.

In this review, we comprehensively summarize the epidemiological evidence of CDI and CRC, analyzing the potential mechanisms underlying the role of *C. difficile* in colonic tumorigenesis. Drawing on established hypotheses in CRC etiology—the alpha-bug, driver–passenger, keystone pathogen, hit-and-run, and common ground hypotheses—we comprehensively elaborate on the potential mechanisms by which *C. difficile* may contribute to CRC development. These proposed mechanisms include toxin-mediated effects, pro-inflammatory responses, immune evasion, metabolic reprogramming, oxidative stress, cellular senescence, biofilm formation, and the production of tumor-promoting microbial metabolites. By integrating existing evidence, our analysis aims to shed light on the multifaceted role of *C. difficile* in CRC pathogenesis, offering insights into this underexplored yet critical relationship.

Mechanisms of *C. difficile* Pathogenesis and Clinical Manifestations

C. difficile (originally named *Bacillus difficile*), a Gram-positive, spore-forming, obligate anaerobic, and toxin-producing bacillus, was first identified as part of the normal intestinal flora of healthy newborn infants by Hall and O'Toole in 1935.[27] In 1978, *C. difficile* was found to primarily cause pseudomembranous colitis.[28] Currently, CDI is the most common and costly healthcare-associated infection. *C. difficile* invades the body as dormant spores and is activated by specific bile salts and amino acids[29–31] in the colon to form vegetative cells. Following antibiotic-induced dysbiosis of the gut microbiota, the metabolically active vegetative cells colonize and infect the colon by producing multiple toxins.[32] Alongside toxin production, *C. difficile* initiates a genetic program for sporulation, thus adopting a dormant and oxygen-tolerant form that enables it to persist within the intestine.[33] *C. difficile* undergoes a complex, dual-phase life cycle, alternating between a highly resilient spore form and a metabolically active vegetative state, thereby perpetuating the vicious cycle of CDI.

The symptoms of CDI mainly correlate with the production of three exotoxins: Toxin A (TcdA), toxin B (TcdB),[34] and *C. difficile* transferase (CDT or binary toxin).[35] Inactivation of Ras homology (Rho) GTPases by TcdA and TcdB in a glucosylation-dependent manner leads to direct cytotoxic effects of actin depolymerization and cell death, leading to alterations in the actin cytoskeleton, disruption of barrier function, and apoptosis[36] [Figure 1]. CDT belongs to the family of binary ADP-ribosylating toxins consisting of two components: CDTa, the enzymatic ADP-ribosyltransferase that modifies actin, and CDTb, which promotes host cell binding, pore formation, and CDTa translocation.[37] CDT ADP-ribosylation alters host cellular morphology, including the detachment of tight junctions connecting epithelial cells, and culminates in cellular rounding and epithelial tissue shedding [Figure 1]. In addition to cytopathic and direct cytotoxic effects in epithelial cells, toxigenic *C. difficile* strains release protein toxins (TcdA, TcdB, and CDT) that also induce an intense local inflammatory response characterized by inflammatory cell infiltration, submucosal neuron activation, secretion of cytokines, chemokines, and arachidonic acid metabolites, and production of substance P and reactive oxygen intermediates.[38]

Under the influence of the aforementioned mechanisms, CDI typically manifests as diarrhea, colitis, or both in susceptible individuals. Mild cases are characterized by diarrhea and pseudomembranous colitis, while fulminant cases may progress to toxic megacolon and even death.[39] Each acute episode of CDI is typically an acute infection characterized by acute inflammation, cytokine release, and epithelial cell damage. Patients with a history of one CDI episode have a recurrence rate of ~15–35%, while those with a history of two episodes have a significantly higher recurrence rate of 33–65%.[40–45] In some cases, patients may experience recurrent episodes over several years.[45–47] Mechanistically, CDI recurrence is not only associated with residual spores but is also closely linked to inadequate immune responses during reinfection and

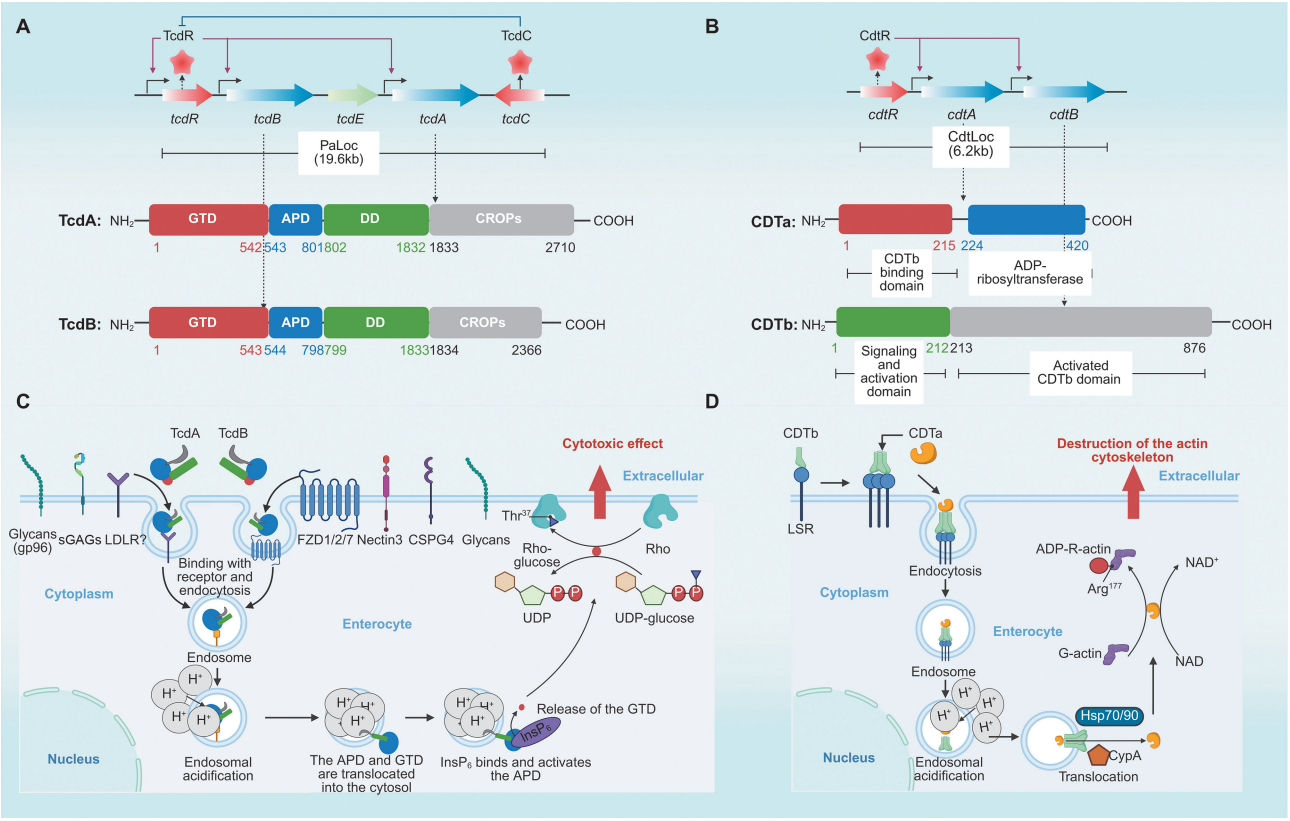

Figure 1: Organization of *C. difficile* toxin genes, and the structure and function of the TcdA, TcdB, and CDT. (A) The schematic representation of the pathogenicity locus (PaLoc) and the primary structure of TcdA and TcdB. The transcriptions of toxin-encoding genes, *tcdA* and *tcdB*, are regulated by the regulatory genes, *tcdR* (positive) and *tcdC* (negative). *TcdE* is involved in the secretion of toxins. The *tcdA* and *tcdB* encode the toxins TcdA and TcdB, respectively, which are organized into four functional domains: The glycosyltransferase domain (GTD; red), the autoprocessing domain (APD; blue), the delivery or pore-forming domain (green), and the combined repetitive oligopeptides domain (CROPS; gray). (B) The schematic representation of the binary toxin locus (CdtLoc) and the primary structure of CDT. The transcriptions of CDT-encoding genes, *cdtA* and *cdtB*, are positively regulated by the regulatory gene *cdtR*. The CDT consists of a binding component (CDTb) and an enzymatic component (CDTa). The former is made up of the CDTb binding domain and the ADP-ribosyltransferase and the latter comprises the signaling and activation domain and the activated CDTb domain. (C) Mechanism of action of TcdA and TcdB. TcdA and TcdB bind distinct surface receptors and then are internalized by receptor-mediated endocytosis. LDLR or LDLR family members have been implicated in TcdA uptake; however, the precise receptor identity remains incompletely defined, and is therefore indicated by a question mark in the schematic. An influx of protons the endosomal acidification, which induces pore formation and translocation of the GTD and APD into the cytosol. Inositol hexakisphosphate (InsP6) binds and activates the APD of the cytoplasm, resulting in the cleavage and release of the GTD. The free GTD glucosylate Rho proteins at threonine 37 by transferring the glucose moiety from UDP-glucose. Glucosylation disrupts GTPase signaling and leads to cytotoxic effects and apoptotic cell death. (D) Mechanism of action of CDT. The monomeric form of CDTb binds to the lipolysis-stimulated lipoprotein receptor (LSR). CDTb undergoes proteolytic activation and oligomerization on the cell surface, which facilitates the binding of CDTa to the prepore–receptor complex. The receptor-CDT complex is subsequently internalized into cells. Endosome acidification triggers conformational changes in CDTb and the translocation of CDTa into the cytosol, which is facilitated by heat shock protein 70 (Hsp70), Hsp90, and cyclophilin A (CypA). In the cytosol, CDTa ADP-ribosylates actin at arginine-177. ADP-ribosylation of actin results in the eventual breakdown of the actin cytoskeleton and cytopathic cell rounding. APD: Autoprocessing domain; CROPs: Combined repetitive oligopeptides domain; CSPG4: Chondroitin sulfate proteoglycan 4; CypA: Cyclophilin A; *cdtA*: *C. difficile* transferase A; *cdtB*: *C. difficile* transferase B; *cdtR*: *C. difficile* transferase R; DD: Delivery domain; FZD: Frizzled; GTD: Glycosyltransferase domain; HSP70/90: Heat shock protein 70/90; InsP6: Inositol hexakisphosphate; LDLR: Low-density lipoprotein receptor; LSR: Lipolysis-stimulated lipoprotein receptor; NAD: Nicotinamide adenine dinucleotide; PaLoc: Pathogenicity locus; Rho: Ras homology; sGAGs: Sulfated glycosaminoglycans; *tcdA*: Toxin A; *tcdB*: Toxin B; *tcdC*: Toxin C; *tcdE*: Toxin E; *tcdR*: Toxin R; UDP: Uridine diphosphate.

persistent disruption of the normal colonic microbiota.[48] The high recurrence rate of CDI indicates that *C. difficile* has a persistent impact on the gut.

Tumorigenesis is generally a long-term process driven by chronic and repeated insults rather than a single event. The high recurrence rate of CDI and the refractory nature of fulminant cases observed in clinical practice may prolong the impact of acute CDI on the gut,[49,50] possibly leading to chronic inflammation or persistent dysbiosis, which are established risk factors for CRC.[51,52] These effects might contribute to the malignant transformation of IECs and CRC development. However, there is currently no direct evidence of a clear causal link between recurrent or fulminant CDI and CRC. Sparse clinical cohort studies have evaluated this relationship and provided an epidemiological link between CDI and human CRC incidence (see next section). Nevertheless, research on the mechanisms of *C. difficile*-induced colorectal carcinogenesis remains limited, resulting in an underestimation of the potential causal link between CDI and CRC.

Epidemiological Association Between CDI and CRC

In the twentieth century, CDI incidence increased, becoming a well-publicized cause of hospital-acquired infection in developed countries.[53] Additionally, the global epidemiology of CRC has fluctuated significantly. Since the 1990s, the incidence of CRC among individuals <50 years has been increasing.[54,55] These parallel trends in epidemiology suggest a strong link between CDI and CRC.

Currently, *C. difficile* is considered an opportunistic pathogen during cancer treatment. Thus, immunocompromised

patients with CRC are at a higher risk for CDI. CRC predisposes individuals to CDI through various interconnected pathways, including alterations in gut microbiota composition and diversity. These alterations disrupt the delicate balance between commensal and pathogenic bacteria, thereby facilitating the colonization and proliferation of *C. difficile*.[56] Nevertheless, limited data exist on the fecal carriage and intestinal colonization of *C. difficile* in patients with CRC. The abundance of *C. difficile* is reportedly high in preoperative patients with CRC and is not hospital-acquired. Patients with advanced CRC, characterized by lymph node metastasis, have a higher rate of *C. difficile* colonization. Meanwhile, patients with CRC who test positive for stool occult blood have lower rates of *C. difficile* colonization than those who test negative.[23] In addition, the proportion of CRC patients with anti-TcdB IgG levels above the threshold (66.7%) is significantly higher than that of healthy individuals (30.8%).[24] Additionally, the prevalence of *C. difficile* in cancerous tissues is 60%, compared to only 20% in adjacent non-cancerous tissues[25] [Figure 2]. These limited studies indicate high colonization of *C. difficile* and prevalence of CDI in patients with CRC.[26] A large multinational cohort study indicated that cancer patients have a 15.6% CDI recurrence rate within 90 days, which increases with subsequent episodes. Among these, female gender, dialysis, and vancomycin treatment elevate the risk of CDI recurrence.[57] While a consensus acknowledges the association between CRC and a high incidence of CDI, a direct causal relationship remains debatable in humans. Therefore, it is unknown whether *C. difficile* dysbiosis precedes or follows the development of colorectal neoplasia.

The association between CDI and subsequent CRC has been sporadically revealed by epidemiologic studies. A large-scale longitudinal cohort study found an increase (about 2.7-fold) in CRC diagnosis in the first 4 years following CDI (3.11 per 1000 person-years) compared to the non-CDI (1.16 per 1000 person-years). This filled the epidemiological gap regarding the potential association between *C. difficile* and the risk of colon cancer.[58] Another study observed a higher incidence of CRC in obese patients with CDI than in obese patients without CDI[59] [Figure 2]. Additionally, *C. difficile* was identified as an excess body weight-CRC-specific signature.[60] These study results provide important and new epidemiologic evidence supporting the hypothesis that *C. difficile* is associated with an increased risk of subsequent CRC.

The relationship between CDI and oncogenesis appears unambiguous, and the knowledge of *C. difficile*'s association with colorectal neoplasia has important clinical implications. However, the timing of *C. difficile* dysbiosis relative to cancerous onset is incomprehensible in humans, even in studies of clinical cohorts. Further in-depth and large-scale cohort studies are needed to clarify their complex relationship. The cause-effect relationship between CDI and CRC relies mainly on data from experimental

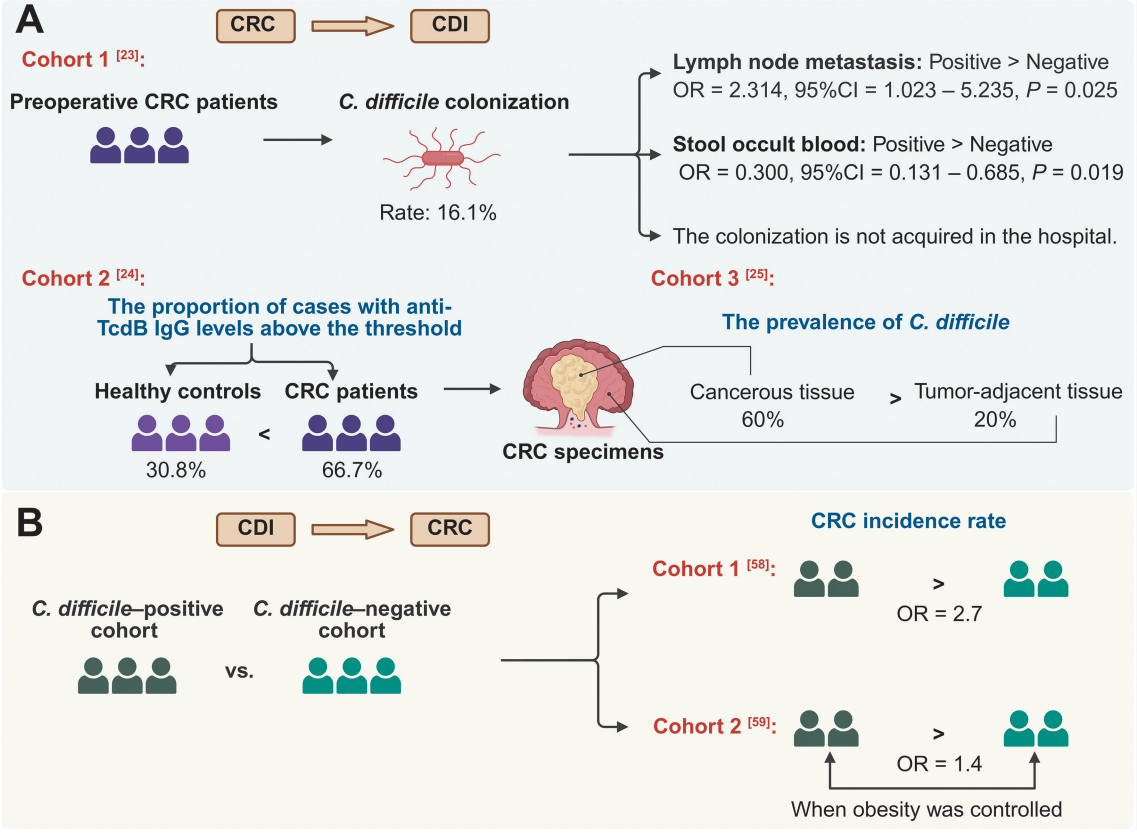

Figure 2: Epidemiological association between CDI and CRC. (A) The existing cohort studies supported those patients with CRC were susceptible to CDI. (B) The existing cohort studies have shown that the incidence of CRC tends to increase following a CDI. CDI: *C. difficile* infection; *C. difficile*: *Clostridioides difficile*; CRC: Colorectal cancer; OR: Odds ratio.

models. Therefore, exploring the various interactions between the colonic mucosa and *C. difficile* is essential to understanding how *C. difficile* might influence malignancy development. Next, we will present experimental data that examine the etiological *vs*. non-etiological roles of *C. difficile* in CRC and the underlying mechanisms. This will help to address the core question of whether *C. difficile* is a cause or a consequence of CRC.

Microbial Hypotheses in CRC Etiology

CRC occurrence is related to changes in the overall intestinal flora structure and infection with one or several specific bacteria. To better understand the relationship between CDI and CRC, we need to introduce the hypothesis model that explains the role of microorganisms in CRC [Figure 3].

Alpha-bug hypothesis

Cynthia L. Sears and Drew M. Pardoll formulated the alpha-bug hypothesis in 2011. "Alpha-bugs" are certain intestinal commensal bacteria that induce epithelial gene mutations directly and indirectly. The indirect effect involves reshaping the colon's bacterial community and promoting mucosal immune responses by alpha-bugs, resulting in CRC.[61] For example, enterotoxigenic *Bacteroides fragilis* (ETBF) is considered a pro-tumorigenic alpha-bug, which induces carcinogenesis in IECs by secreting a toxin and promoting intestinal mucosal inflammation[62] [Figure 3]. "Alpha-bugs" bacteria may enhance carcinogenesis by selective "crowding out" of cancer-protective intestinal bacteria. *C. difficile*, as a causal agent driving excessive host inflammatory responses[63] and altering the gut microbiome community,[64] may be a potential alpha-bug in the pathogenesis of intestinal neoplasms.

Driver–passenger hypothesis

The alpha-bug model does not explain the increased abundance of undifferentiated gut microflora in the early stage of CRC progression. The novel driver–passenger hypothesis, introduced by Harold Tjalsma in 2012,[65] explains the complex changes in the intestinal microbiota associated with CRC [Figure 3]. According to this model, indigenous intestinal bacteria (defined as driver bacteria, such as *Enterococcus faecalis* [*E. faecalis*], some *Escherichia coli* [*E. coli*] strains, and *Bacteroides fragilis* [*B. fragilis*]) trigger DNA damage in epithelial cells, contributing to cancer initiation. Next, the ongoing tumorigenesis alters the surrounding microenvironment, favoring the proliferation of opportunistic bacteria (termed bacterial passengers, such as *Streptococcus bovis/gallolyticus* and *Clostridium septicum*). Thus, this model suggests that disease progression allows bacteria in the

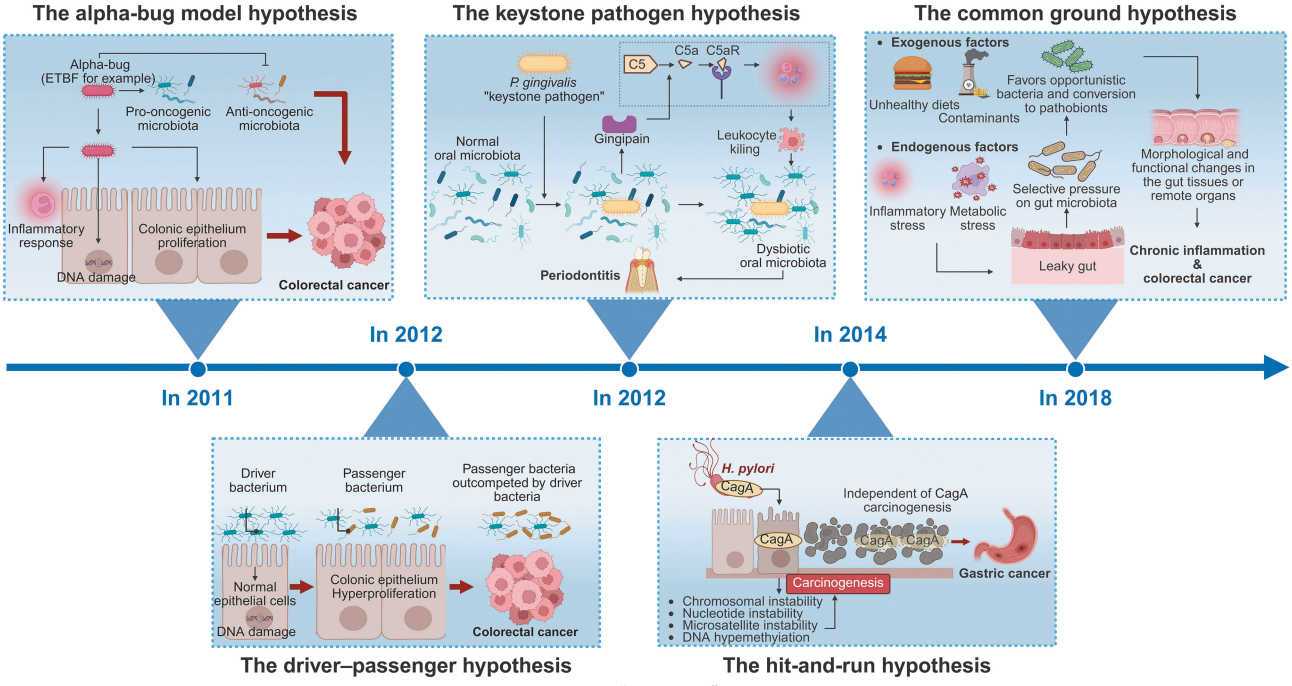

Figure 3: The hypothesis models associated with CRC. The alpha-bug hypothesis: The "Alpha-bugs" bacteria, such as enterotoxigenic *Bacteroides fragilis* (ETBF), can directly induce carcinogenesis in intestinal epithelial cells. This is achieved by promoting intestinal mucosal inflammation and reshaping the colon's bacterial community, which may lead to a dysbiotic state conducive to cancer development. The driver–passenger hypothesis: This hypothesis proposes that driver bacteria can trigger DNA damage in epithelial cells, which contributes to cancer initiation. The ongoing tumorigenesis alters the surrounding microenvironment, thus favoring the proliferation of passenger bacteria, further contributing to the carcinogenesis process. The keystone pathogen hypothesis: *Porphyromonas gingivalis* induces periodontitis through the bacterium's gingipain at very low colonization levels, accompanied by significant alterations in the number and community organization of the oral commensal bacteria. Those pathogens, which are in low abundance but have large effects, are referred to as "keystone species". The hit-and-run hypothesis: This hypothesis suggests that *Helicobacter pylori* infection can lead to gastric cancer through mechanisms, such as chromosomal instability, nucleotide instability, and microsatellite instability, persisting even after the bacterium has been eliminated. The common ground hypothesis: This hypothesis posits that a combination of exogenous factors, such as unhealthy diets and contaminants, and endogenous factors, like inflammatory and metabolic stress, can facilitate the transformation of opportunistic bacteria into pathogens. This transformation leads to selective pressure on the gut microbiota and induces morphological and functional changes in the gut tissues. Consequently, these changes may precipitate chronic inflammation and colon cancer. CRC: Colorectal cancer; CDI: *C. difficile* infection; C5: Complement component 5; CagA: Cytotoxin-associated gene A.

tumor microenvironment (TME) to outcompete other bacteria that can promote carcinogenesis. *C. difficile*, a well-known opportunistic pathogen highly susceptible to antibiotic-induced dysbiotic environmental changes,[66] is more prone to act as a "passenger" bacterium.

Keystone pathogen hypothesis

In ecology, the term "keystone" describes individual species with disproportionately large effects on their communities. The "keystone pathogen" hypothesis raised by Curtis *et al*[67] holds that certain low-abundance microbial pathogens can cause disorders by remodeling a normally benign microbiota into a dysbiotic one [Figure 3]. For instance, the presence of ETBF in animal models at low abundance levels (<1–2% of the colonic microbiota) suggests that ETBF may act as a keystone pathogen in colon tumorigenesis.[61] Similarly, *C difficile*, which has a low abundance in the microbial community, alters the host microbiota structure through its toxin-mediated inflammatory response and destruction of the intestinal microbiota. This triggers or exacerbates colitis[68] and induces intestinal malignancy.[55] Overall, the keystone pathogen hypothesis offers a valuable perspective on the role of specific microbial species in CRC development.

Hit-and-run hypothesis

In CRC, the hit-and-run hypothesis has been mainly considered in relation to the presence of certain viruses.[69] However, the hypothesis also describes a scenario where temporary colonization and damage by a tumorigenic bacterium lead to tumorigenesis. This theory has been applied to *Helicobacter pylori* infections in gastric cancers, where the pro-oncogenic action of the bacterial toxin cytotoxin-associated gene A (CagA) results in genetic and epigenetic alterations[70] [Figure 3]. It suggests that transient colonization and damage by a pro-carcinogenic bacterium can be necessary and sufficient to drive tumorigenesis. We highlighted that *C. difficile* multiplies in the gut, producing toxins that cause inflammation and damage (the "hit" phase). Persistent damage and inflammatory response caused by the previous toxins may lead to a long-term imbalance of the gut microbiota even if *C. difficile* is cleared by antibiotic treatment or disappears naturally from the gut (the "run" phase). The hit-and-run hypothesis suggests that the microbiome might be responsible for more cancers than previously hypothesized, such as *C. difficile*-induced carcinoma.

Common ground hypothesis

Microbiota dysbiosis and intestinal barrier impairment are associated with the development of various chronic inflammatory disorders and colitis-associated cancers. An emerging speculation of common factors involved in the pathogenesis of chronic disorders has been proposed as the "common ground hypothesis[71]" [Figure 3]. This hypothesis supposes that exogenous and endogenous factors create a leaky gut, making the gut lining highly permeable to pathogens, potentially leading to chronic inflammation and cancer. Following the "common ground hypothesis," microbial dysbiosis and intestinal barrier impairment are at the core of the chronic inflammatory process associated with IBD-CRC.[72] The impairment of intestinal barrier function or disruption of mucosal T cells by inflammatory mediators in patients with IBD favors microbial dysbiosis, such as *C. difficile* colonization.[73] Thus, this evidence further shows a correlation among *C. difficile*, inflammation, and cancers based on shared aberrant epithelial innate immune responses. Overall, understanding the core interplay between gut microbiota and host barriers based on the common ground hypothesis at the early subclinical phase will shed light on novel therapeutic approaches for cancers caused by chronic inflammatory disorders.

Subsequently, Garrett *et al*[74] outlined three distinct theoretical models that elucidate how microbes or microbial communities might contribute to CRC development. Model one focuses on the role of individual microbes, emphasizing the direct carcinogenesis effect of specific bacteria in CRC, which aligns with the alpha-bug model and the keystone pathogen hypothesis. Model two considers the collective impact of the microbial community as a whole. This model is similar to the driver–passenger and common ground hypotheses, emphasizing the importance of the collective behavior of microbiota in CRC development. Finally, model three proposes a more nuanced view, suggesting that individual microbes and microbial communities can play a role in CRC development, potentially through sequential collaborations. Thus, we support the hypothesis that the synergistic actions of *C. difficile* and other microbiota members are key to colon oncogenesis rather than acting independently to induce colon tumors. These models provide a comprehensive view of the potential mechanisms by which the microbiota can influence CRC, recognizing the dynamic interplay among individual microbes, microbial communities, and the host.

CDI and CRC: Hypothesized Etiological Mechanisms

Tumor formation is a complex process involving multiple signaling and molecular alterations. Recently, Dr. Douglas Hanahan outlined 14 functional capabilities or hallmarks of cancers.[51,52] Research suggests that gut microorganisms, especially bacteria, are involved in each hallmark of CRC and contribute to pathogenesis and treatment.[75,76] Nevertheless, the pathogenesis of microorganism-related CRC results from multiple mechanisms.

If CDI and its recurrence can represent a risk for CRC induction, it is important to identify the possible underlying mechanisms. The other big question in the current topic is, "what mechanisms does *C. difficile* employ to induce, promote, and progress the development of neoplastic lesions?" Recently, evidence has accumulated, and we have many albeit indirect but important elements that suggest a possible pathogenesis mechanism. The potential for *C. difficile* to trigger CRC will be meticulously examined through the following aspects: Toxic effects, proinflammatory responses, immune evasion, metabolic reprogramming, oxidative stress, cellular senescence, biofilm formation, and metabolites [Figure 4]. This analysis offers a reasonable extrapolation of how *C. difficile* might cause colorectal neoplasia, expanding our understanding

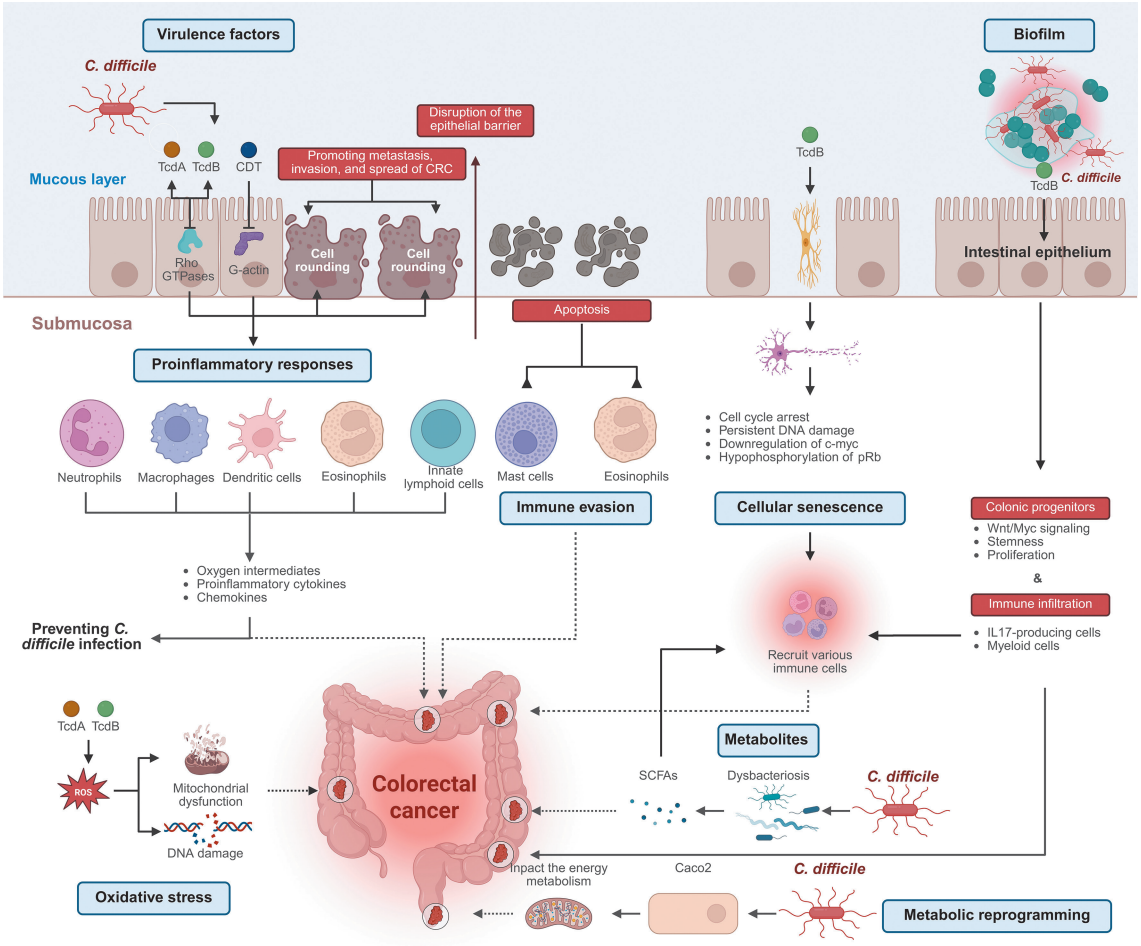

Figure 4: The hypothesized mechanisms of the etiological association of *C. difficile* with CRC. Based on the pathogenesis of *C. difficile* colitis and the existing evidence of bacterial carcinogenesis, we have reasonably extrapolated the pathogenesis of *C. difficile* carcinogenesis. The involved pathogenic mechanism by which bacteria lead to CRC involves many aspects, including inflammation, pathogenic bacteria, and their virulence factors, genotoxins, oxidative stress, bacterial metabolites, and biofilm. The solid arrows in our model represent proven experimental evidence, while the dashed arrows indicate our reasonable conjectures that require further experimental validation. CRC: Colorectal cancer; CDT: Cytolethal distending toxin; *C. difficile*: *Clostridioides difficile*; ROS: Reactive oxygen species; SCFA: Short-chain fatty acids; TcdA: Toxin A; TcdB: Toxin B.

of the carcinogenic mechanisms of known CRC candidate pathogens.

Toxic effects

Bacterial toxins that can cause changes to the genetic material within host cells (such as double-strand DNA breaks,[77] covalent DNA modification,[78] mutations,[79] chromosomal aberrations,[80] and genomic instability[81]) are called genotoxins. This genetic damage in somatic cells could result in somatic mutations, potentially leading to the malignant transformation of the cell and the development of cancer. Currently, three bacterial genotoxins have been identified. Two are protein toxins: The cytolethal distending toxin family produced by many Gram-negative bacteria[82] and the typhoid toxin produced by *Salmonella enterica* serovar Typhi.[83,84] The third member, colibactin, is a peptide-polyketide genotoxin produced by strains belonging to the phylogenetic group B2 of *E. coli*.[79,85] Thus, genotoxins are crucial in CRC initiation and progression. Although there is no direct evidence that *C. difficile* toxins (TcdA, TcdB, and CDT) act as classical genotoxins causing DNA or chromosomal damage in host IECs, they may indirectly impair genetic material by generating reactive oxygen species (ROS).[86] Increased ROS interact with vulnerable cellular components, such as lipids, proteins, and DNA, further deregulating oncogenic signaling pathways that play a role in CRC development.[87] This potential role of oxidative stress in *C. difficile*-associated CRC is extensively explored in the section "Oxidative stress".

In addition to the potential alteration of genetic material, *C. difficile* toxins disrupt IEC cytoskeleton, leading to changes in cell morphology. Mechanistically, TcdA and TcdB inactivate host GTPases and CDT irreversibly, disrupting or rearranging the host cell cytoskeleton and causing cytopathic effects.[38] Deregulation of Rho GTPases has been linked to many "hallmarks of cancer," including oncogenic transformation, cell survival, and tumor metabolism and metastasis. GTPase has a suppressor or negative modifier function in stress-associated processes, such as cancer.[88] A previous study revealed that Rho function loss in the thymus is accompanied by thymic lymphoma development,[89] which is related to inactivation of GTPase, leading to the continuous activation of downstream signaling pathways, uncontrolled

cell proliferation, and carcinogenesis.[90] Additionally, cell cytoskeleton remodeling, mostly in actin structure, highly influences cell mechanical properties, force generation, and adhesion potentials. Notably, the invasive nature of cancer cells is correlated with their increased traction force, high deformability, and altered cell adhesion.[91] Hence, the disruption of cell cytoskeleton by *C. difficile* toxin may be crucial for CRC invasion and metastasis. Additional, *C. difficile* toxins also exert cytotoxic effects by inducing apoptosis in IECs and disrupting intercellular junctions, thereby compromising epithelial integrity and leading to long-term impairment of the intestinal barrier.[92,93] Impaired mucosal repair can exacerbate the persistent damaging effects of CDI. Compromised epithelial barrier facilitates the translocation of pathogenic bacteria and their toxins into the mucosal layer, increasing exposure to carcinogenic agents, such as *E. coli* and its virulence factors.[94] This can further stimulate the immune system, amplifying inflammatory responses and increasing the risk of carcinogenesis. This disruption creates a favorable environment for tumorigenesis, thereby enhancing susceptibility to tumor development.

As illustrated above, *C. difficile* toxins can potentially promote CRC development both indirectly, by generating ROS that damage genetic material, and directly, by disrupting the cytoskeleton of IECs. These actions not only disrupt the normal functioning of IECs but also create a microenvironment that is permissive for tumor development. Over time, these combined effects may significantly increase the risk of CRC development. Thus, gaining a deeper understanding of the complex interplay between toxin-induced cellular changes and the occurrence and progression of CRC is essential for elucidating their modulatory role in CRC pathogenesis.

Proinflammatory responses

German physician Rudolf Virchow first observed the link between inflammation and tumor growth in the nineteenth century, describing leukocyte infiltrates within tumors.[95] Proinflammatory responses have now become a common hallmark of tumors.[51,52] Several intestinal pathogens induce tumorigenesis by promoting inflammatory responses, such as ETBF[62,96] and *Fusobacterium nucleatum* (*F. nucleatum*).[97] Inflammatory microenvironments caused by aberrant intestinal microbiota are undoubtedly important in supporting the growth and survival of intestinal neoplasms.

C. difficile toxin-induced damage to the intestinal barrier initiates the recruitment of circulating immune cells to the site of infection, including neutrophils, macrophages, and dendritic cells (DCs), instigating innate and adaptive immune responses. Neutrophils are crucial in pseudomembranous colitis and produce bactericidal reactive oxygen intermediates,[98] proinflammatory cytokines,[99,100] and chemokines,[101,102] leading to an intense neutrophil-mediated inflammatory response. Monocytes, macrophages, and DCs are major sources of inflammatory cytokines and secrete tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6, IL-8, prostaglandin E2 (PGE2), and leukotriene B4 upon exposure to TcdA and TcdB.[103–105] TcdA could mediate nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) phosphorylation and TNF-α expression in macrophages[106] and induce macrophage inflammatory protein 1α secretion,[107] leading to inflammatory responses and tissue damage in the human colon. DCs also play an important role in regulating inflammation during CDI. Surface layer proteins[108,109] and toxins[110,111] could promote DC maturation and chemokine expression, including C-X-C motif chemokine ligand 2 (CXCL2), IL-12, TNF-α, and IL-10.

A recent study illustrated the role of inflammation driven by *C. difficile* in carcinogenesis. Drewes *et al*[55] demonstrated a surprising role of inflammatory response for *C. difficile* in driving CRC. Culturing and reassociation experiments revealed that toxigenic strains of *C. difficile* drive the tumorigenic phenotype of a subset of patients with CRC-derived mucosal slurries in germ-free $Apc^{Min/+}$ mice. Mechanistically, CRC tumorigenesis is dependent on TcdB, leading to a pro-carcinogenic mucosal immune response characterized by IL-17 production from multiple cellular sources and prominent myeloid infiltration. *C. difficile*-induced tumors occur with concomitant activation of myeloid and IL-17-producing lymphoid cells, reminiscent of those observed after ETBF infection in the same model.[112] This data support the role of inflammation caused by toxigenic strains of *C. difficile* as a driver of tumorigenesis, highlighting the complex relationship between *C. difficile* and CRC.

The experimental models provide evidence that aberrant innate immune responses in epithelial cells are involved in the pathogenesis of colitis and tumor development. This further supports the correlation among *C. difficile*, inflammation, and intestinal neoplasms, providing strong evidence that *C. difficile* acts as a tumor promoter, consistent with the "common ground hypothesis." Future in-depth studies will elucidate how chronic inflammation caused by CDI induces and promotes CRC development.

Immune evasion

Microbes trigger and reinforce proinflammatory immune circuits and also elicit or exploit immunosuppressive responses, which can impair antitumor immunity and promote the carcinogenesis of colonic epithelial cells (CECs).[113] For example, invasive *F. nucleatum* interacts with immune cells in the colon mucosa, leading to decreased T cell density,[114,115] enhanced M2 macrophage polarization,[116] NK cell cytotoxicity inhibition,[117,118] and elevated tumor-associated neutrophils[97] that diminish anti-tumor immunity. ETBF could induce the signal transducer and activator of transcription 3 (STAT3) pathway and the Th17 cancer pathway by promoting the local infiltration of regulatory T cells, which establishes an immunosuppressive TME and contributes to CRC development.[119] In conclusion, in the process of carcinogenesis, gut microbes suppress immunosurveillance against abnormal cells and contribute to the malignant transformation of intestinal tumors.

C. difficile toxin-induced damage to the intestinal barrier usually provokes an inflammatory response characterized

by the recruitment and activation of immune cells. However, there is also evidence that *C. difficile* can activate immunosuppression. Like other bacteria, the common immune evasion strategy of *C. difficile* involves the production of toxins that inactivate Rho-family GTPases,[120] which could disrupt phagocyte immune functions[121] and inhibit the secretory response of human mast cell line-1.[122] Phagocytes and mast cells are unique tissue-resident immune cells that can provoke anti-tumor immune response.[123,124] Additionally, one recent research found that *C. difficile* is closely associated with the identified tumor immune subtypes from gastrointestinal tumor samples, with higher levels in subtype 2 than in subtype 1 ($P = 0.015$).[125] The immune subtype 2 is closer to the immune-desert subtype, namely, almost no immune cell infiltration.[126] Therefore, *C. difficile* is speculated to be closely associated with immunosuppressive TME. A putative mechanism shows that the activity of the binary toxin CDT is associated with the suppression of the otherwise protective colonic eosinophilic host responses *in vivo*.[127] Studies have shown that suppressing protective colonic eosinophilia can enhance *C. difficile* virulence[128] and induce carcinogenesis via immune evasion.[129] Thus, it is speculated that *C. difficile* may promote CRC growth by facilitating the evasion of the host immune response through the promotion of an immunosuppressive TME. Understanding the immune responses of *C. difficile* to the host is crucial for elucidating the pathogenic mechanisms of immune evasion in CRC.

In summary, *C. difficile* can trigger inflammation while also inducing immune evasion, which disrupts the balance of host immune system. Immune dysregulation plays a crucial role in creating a tumor-promoting microenvironment.[130,131] Under normal conditions, the immune system detects and eliminates abnormal cells through immune surveillance. However, when this system is compromised—due to chronic inflammation, immune suppression, or immune evasion—precancerous cells may escape detection and proliferate. Persistent immune activation can also lead to the continuous release of pro-inflammatory cytokines, such as IL-6 and TNF-α, which activate oncogenic pathways like NF-κB and STAT3, promoting cell proliferation and inhibiting apoptosis.[132] Additionally, immune tolerance mechanisms, such as the activation of immunosuppressive cells, further allow tumor cells to evade immune clearance.[130,133] Consequently, the diversity, complexity, and dynamic interplay between *C. difficile* and host immune cells pose challenges to studying the carcinogenic effects of CDI.

Metabolic reprogramming

Cancer cells require more energy for rapid growth and metastasis than normal cells. This process is called metabolic reprogramming. Disruption of energy metabolism, especially abnormal glycolysis, is a hallmark of cancer.[51] Intestinal microbiota disorder and their metabolites have been shown to enhance tumor glucose metabolism and oncogenesis by targeting key components of the glycolysis pathway.[134,135] In addition to glycolysis, gut microbiota also regulates lipid metabolic reprogramming in CRC. For example, Colibactin-producing *E. coli* can induce tumor heterogeneity in lipid reprogramming, which in turn facilitates the progression of CRC and chemoresistance.[136] However, there is also a paucity of research on the involvement of intestinal bacteria in the metabolic reprogramming of CRC, necessitating further exploration.

The role of *C. difficile* in host cellular energy metabolism is rarely documented in scientific literature. Extracellular ATP engages purinergic receptor P2X purinoceptor 7 (P2X7) signaling, leading to nucleotide-binding oligomerization domain-like receptor protein 3-independent inflammasome activation and pyroptosis during CDI,[137] linking host energy metabolism with CDI pathogenesis. However, in an *in vitro* anaerobic–aerobic CDI model, *C. difficile* inhibited energy metabolism, including glycolysis and the tricarboxylic acid cycle, and downregulated the electron transport chain in Caco-2 cells during the early stage of infection.[138] Thus, there is limited definite evidence to clarify the interaction between *C. difficile* and metabolic signaling pathways. The role of metabolic reprogramming driven by *C. difficile* in CRC communication requires further investigation to better understand CRC pathogenesis.

Oxidative stress

Under sustained environmental stress, prolonged ROS production can cause significant damage to cellular structures and functions. This damage may induce somatic mutations and lead to neoplastic transformation.[139] *B. fragilis* reportedly promotes CRC occurrence by upregulating spermine oxidase (SMO), leading to SMO-dependent ROS production, which further leads to DNA damage and induces carcinogenesis.[140] Some bacteria induce colon cancer by producing numerous extracellular ROS to change the extracellular oxidative environment, such as *E. faecalis*. Bacteria play an extremely important role in CRC development and occurrence by acting on the reduction–oxidation pathway.

Oxidative stress pathways are reportedly activated or upregulated in response to *C. difficile* TcdA,[98,141,142] and reactive oxygen intermediate generation is independent of Rho inactivation, which involves nuclear translocation of NF-κB before IL-8 release.[143] Furthermore, TcdB induces necrotic apoptosis, which depends on the assembly of the host epithelial cell NOX complex and ROS production. These ROS accumulate at high levels, causing cell death through DNA damage, lipid peroxidation, protein oxidation, and/or mitochondrial dysfunction.[144,145] The cumulative effect of these genetic and epigenetic alterations may eventually lead to CRC development. Additionally, ROS can influence various signaling pathways, such as Wnt/β-catenin and mitogen-activated protein kinase (MAPK),[146,147] which are critical for malignant cell proliferation and survival.[148] In the context of CDI, TcdB induces ROS production, activating Wnt signaling and promoting tumorigenesis in colorectal tissues.[55]

Elevated ROS levels can induce oxidative stress, leading to DNA damage, chronic inflammation, and signaling pathway modulation, all of which contribute to colorectal carcinogenesis.[87] Understanding the multifaceted role of

ROS in CDI-mediated CRC is crucial for developing targeted therapeutic strategies. A multidisciplinary approach is essential to evaluate interventions aimed at mitigating ROS-induced damage, thereby potentially reducing CRC incidence associated with CDI.

Cellular senescence

Cellular senescence is considered a stress response characterized by irreversible cell cycle arrest.[149] When cells accumulate excessively during cellular senescence, it contributes to various diseases, such as inflammation, aging processes, and malignancy.[150] Several hallmarks of aging (e.g., genomic instability, epigenetic alterations, chronic inflammation, and dysbiosis) are very similar to specific cancer hallmarks. In contrast, other features of aging (e.g., telomere attrition and stem cell exhaustion) are likely to suppress oncogenesis.[151] Thus, the role of cellular senescence in carcinogenesis is likely dual. It can play a protective anti-tumor role, while accumulation and persistence are associated with a poor prognosis of CRC. The gut microbiota[152,153] and their metabolites (e.g., butyrate[154] and deoxycholic acid [DCA])[155] can also elicit cellular senescence and tumor development by secreting senescence-associated secretory phenotype. This intriguing link between senescence induced by gut pathogenic bacteria and cancer progression is currently the subject of active research.

Enteric glial cells (EGCs), one of the major cell types in the enteric nervous system, play a crucial role in maintaining the physiological functions of the intestine, encompassing nutrient absorption, barrier integrity, and immune modulation.[156] Emerging data suggest that EGCs in the gut contribute to the progression and metastasis of CRC by influencing inflammation and modulating the TME.[157] Studies have found that EGCs are susceptible to *C. difficile* toxin, which predominantly induces death by apoptosis/necrosis.[158–160] Fettucciari *et al*[161] further demonstrated that TcdB induces senescence in EGCs, mainly triggering irreversible cell cycle arrest, persistent DNA damage, downregulation of c-myc, and hypo-phosphorylation of phosphorylated retinoblastoma protein. Senescent cells cause chronic inflammation that may promote carcinogenesis.[113] Based on these results, it is hypothesized that EGCs that survive TcdB, once they have acquired a senescence state, could cause CRC owing to persistent inflammation, transfer of senescence status, and stimulation of pre-neoplastic cells. A prior review detailed that *C. difficile* TcdB induces senescence and may be a new pathologic player in CRC.[162]

While these observations suggest diverse roles of cell senescence in regulating cancer initiation and progression, the detailed mechanisms of cell senescence induced by *C. difficile* remain largely obscure. Innovative approaches and advanced technologies are needed to uncover these related mechanisms, thereby supporting cellular senescence induced by *C. difficile* as a potential target for CRC therapy.

Biofilm formation

Biofilms are communities of microorganisms enclosed within a self-constructed matrix of extracellular polymeric substances.[163] These biofilms invade the colonic mucosal layer, establishing direct contact with mucosal epithelial cells. This is an emerging concept in studying the relationship between the intestinal microbiota and CRC.[164] One research group found that invasive polymicrobial bacterial biofilms are consistent features of right-sided (proximal) but not left-sided (distal) CRC.[165,166] This may be attributed to the difference in microbiota composition, molecular characteristics, response to chemotherapy, and prognosis between right-sided and left-sided tumors.[167] Next, we reported that human colon mucosal biofilms from patients with CRC are carcinogenic in murine CRC models.[55,168] Recent reports also show that carcinogenesis by biofilms is dependent on *C. difficile* and its toxin, TcdB.[55]

The contribution of *C. difficile* biofilms to tumor progression can arise through several mechanisms, including: (1) Induction of chronic inflammation: Dejea *et al*[165] proposed a model where biofilm formation enhanced colonic epithelial permeability, facilitating bacterial antigen translocation and pro-carcinogenic tissue inflammation. *C. difficile* strains, isolated from patients with CRC positive for biofilms, interact directly with the colonic epithelium and are linked to pro-carcinogenic changes involving induction of chronic inflammation.[55] (2) Promotion of metabolome: Host and bacterial metabolites act synergistically to promote biofilm formation and cellular proliferation, creating conditions conducive to oncogenic transformation in CECs. Commensal microbiota enhances biofilm formation by *C. difficile* by converting cholate into deoxycholate.[169] Host-derived mucus promotes *C. difficile* growth, which is related to significant upregulation of central metabolism genes, including sugar uptake, the Wood–Ljungdahl pathway, and the glycine cleavage system. Therefore, we speculate that CECs covered with *C. difficile* biofilms are exposed to significantly higher levels of various metabolites, initiating or aggravating CRC. (3) Inhibition of antitumor immunity: The bacterial biofilm has been suggested to subvert the host immune response by several mechanisms, which include interfering with humoral immunity, secreting toxins to impair immune recognition, and regulating the inflammatory status of recruited myeloid-derived suppressor cells, macrophages, and neutrophils.[170] Polysaccharides, proteins, or eDNA in *C. difficile* biofilms play a key role in CDI pathogenesis, including attachment to the mucosa and avoidance of host defense.[171] It is hypothesized that matrix molecules in *C. difficile* biofilms contribute to antibiotic resistance[172] and tumor formation in the digestive tract.

Overall, these findings implicate colonic bacterial biofilms as contributors to a pro-oncogenic state. According to the "bacterial driver–passenger" hypothesis, biofilms of driver bacteria may create novel ecological niches for passenger bacteria in CRC development, eventually outcompeting the driver organism. However, biofilms formed by anaerobic bacteria, such as *C. difficile*, from the human intestinal tract have been poorly characterized. One of the reasons for this is perhaps the difficulties associated with cultivation and standardization conditions for biofilm formation *in vitro*.[173] Although a connection between the composition of the bacterial biofilm and CRC is

becoming increasingly evident, more research is needed to determine the exact role of *C. difficile* biofilm and its constituting species in carcinogenesis.

Metabolites

Various metabolites possess different properties. Some beneficial metabolites, including ursodeoxycholic acid and butyrate, could impede tumor development and progression,[75] while others, such as primary bile acids and short-chain fatty acids (SCFAs), are harmful and have proinflammatory and carcinogenic properties.[174] For instance, gut microbiota-derived DCA suppressed $CD8^+$ T cell responses by inhibiting Ca^{2+}-nuclear factor of activated T cells 2 signaling, thus promoting CRC growth in mice.[175] Thus, in the process of carcinogenesis, gut microbe-derived metabolites suppress immunosurveillance against abnormal cells and contribute to malignant transformation. In addition to immunosuppression, gut microbiota-derived metabolites also promote CRC development by enhancing cancer cell proliferation, increasing DNA damage, boosting tumor invasion and metastasis, diminishing cell–cell adhesion, and promoting genomic instability.[75,176]

The relationship between metabolites and CDI is highly significant. *C. difficile* could liberate host-derived nutrients through cytotoxic activity and the creation of a pro-inflammatory environment.[68] Previously, we observed a significant reduction in SCFA yield within the intestinal flora of patients with CDI compared to that in the normal control group.[177] Therefore, *C. difficile* may affect the metabolism of SCFAs by changing the distribution of bacteria, and the decreased proportion of SCFAs will cause an immune inflammatory response and induce colon cancer development. Clearly, much is still to be understood about the complex interplay between different gut metabolites and how they may promote *C. difficile* growth and CRC development. Further in-depth and exact mechanistic studies exploring the role of metabolites in the carcinogenic pathogenesis of *C. difficile* are required in the future.

Conclusions and Perspectives

The increase in antimicrobial resistance, high infection rates, and the ability of *C. difficile* to subvert the host immune response have made this pathogen one of the most recurrent infection-causing pathogens.[32] However, the exact mechanisms by which *C. difficile* contributes to human diseases, particularly malignancies, are not fully understood. Drewes *et al*[55] demonstrated a surprising role of *C. difficile* in driving CRC in preclinical models, which provides a novel insight for oncologists and microbiologists. Our analyses further reveal that *C. difficile* possesses distinct pro-inflammatory and pro-carcinogenic properties, including recruitment of immune cells, induction of cytokines and transcription factors, activation of immunosuppressive pathways, and selective adherence to and colonization of tumor tissues. Together, these features suggest that *C. difficile* may contribute to the slow progression of carcinogenesis in colorectal mucosal tissues. However, considering the unparallel trends among *C. difficile* colony-forming units, toxin titers, and tumor burden, the variability in host responses and/or other microbiota members is likely a strong modifier. Thus, this review shows that *C. difficile* is more likely to act as a "passenger" bacterium in CRC rather than a "driver" in the "driver–passenger" hypothesis, as suggested by Tjalsma *et al*.

To understand the epidemiology, mucosal biology, and immunology of persistent *C. difficile* colonization and its relationship with colitis-associated and sporadic CRC, translation into human studies will be imperative. Further studies should aim at spatial localization of *C. difficile* in susceptible populations, interactions with epithelial immunity in humans, the TME milieu, and the effect of other microbial colonies on *C. difficile*, which will have significant consequences for unraveling microbiota-cancer connections. Meanwhile, considering the importance of CDI in CRC etiology, future studies should elucidate potential risk factors for CDI. Further, future studies should focus on the effects of various types of CDI (especially recurrent or fulminant CDI) on colon cancer risk, and consideration should be given to the importance of changing screening guidelines for these patient populations.

Importantly, while these studies have primarily focused on CRC, the findings open new avenues for exploring the broader implications of *C. difficile* colonization and infection across the gastrointestinal tract. Given the shared mucosal environments and immune interactions within the digestive system, it is plausible that *C. difficile* and similar pathogens may influence the pathogenesis of other gastrointestinal malignancies, such as gastric, pancreatic, and biliary tract cancers. Thus, expanding research beyond CRC to investigate the role of *C. difficile* and the gut microbiota in the initiation and progression of diverse gastrointestinal tumors is warranted.

In summary, although the natural history of CDI and its potential role in oncogenesis are not yet fully elucidated, current studies provide a robust framework for understanding how individual bacterial species and the broader microbial ecosystem contribute to CRC and potentially other gastrointestinal cancers. Our proposal that *C. difficile* may act as a novel pro-carcinogenic bacterium in gastrointestinal tumors will hopefully inspire future research aimed at uncovering new mechanisms of microbiota-driven oncogenesis and improving therapeutic strategies across a range of digestive system malignancies.

Acknowledgements

The authors would like to thank Editage (www.editage.cn) for English language editing.

Conflicts of interest

None.

Funding

This work was supported by grants from the National Key Research and Development Program of China (Nos.

2022YFC2009801 and 2022YFC2009805), Scientific and Technological Personnel Lifting Project in Hunan Province (No. 2023TJ-Z11), Changsha Science and Technology Plan Project (No. kq2202059), and Innovation and Technology Commercialization Fund of Xiangya Hospital, Central South University (No. 2024ZHJJ06) and the National High-Level Medical Talents—National Excellent Young Physician Program.

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How to cite this article: Zou J, Tian BY, Xiao YY, Wu AH, Li CH. *Clostridioides difficile*: A suspected pro-carcinogenic bacterium for gastrointestinal tumors. Chin Med J 2026;139:7–22. doi: 10.1097/CM9.0000000000003834