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Perspective

# *Clostridium difficile* as a potent trigger of colorectal carcinogenesis

**Javad Nezhadi[1,2] · Masoud Lahouty[3] · Mohammad Ahangarzadeh Rezaee[2] · Manouchehr Fadaee[4]**

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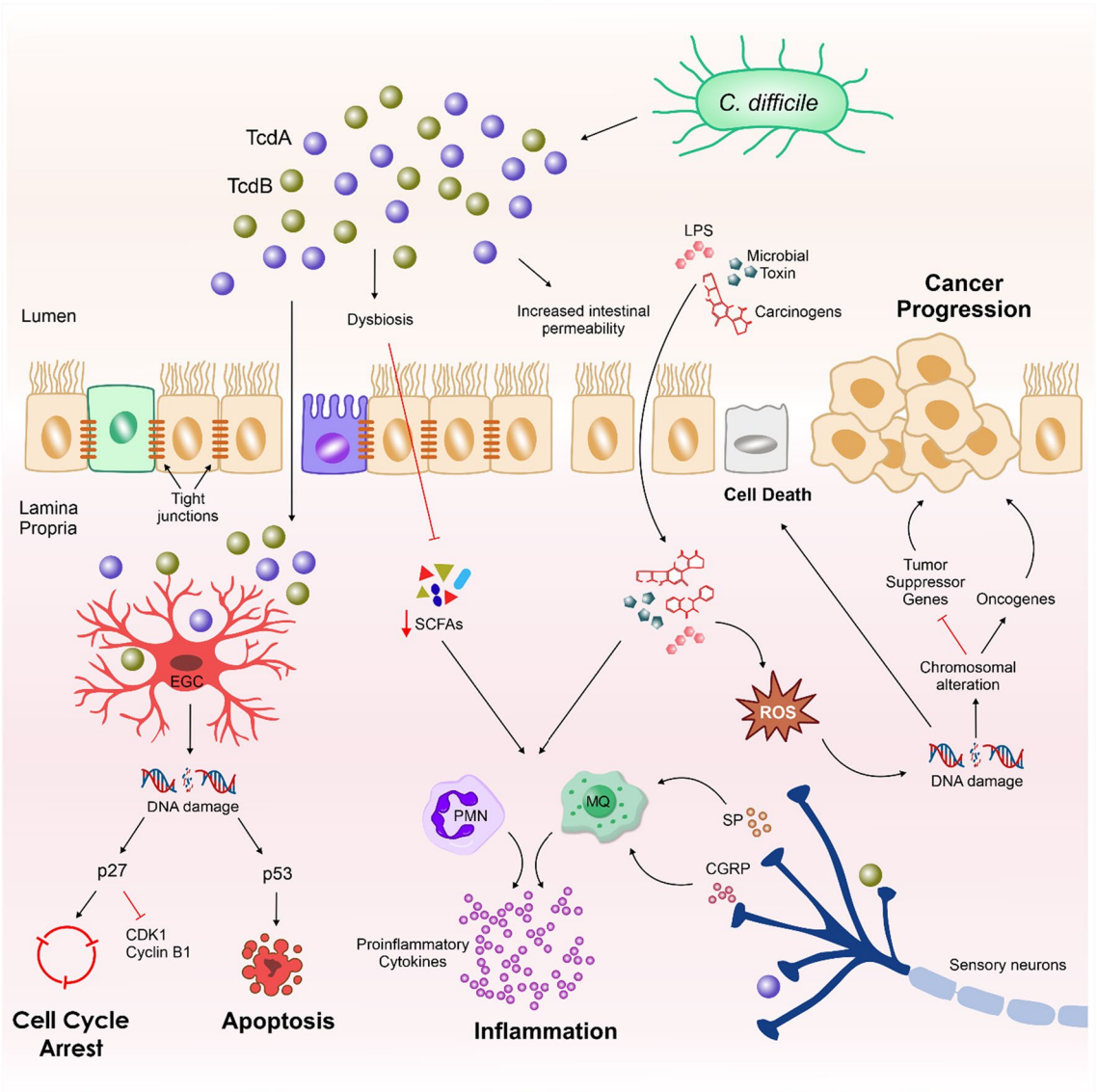
**Abstract**
*Clostridium difficile*, traditionally recognized as a cause of antibiotic-associated colitis, has emerged as a potential oncogenic factor in colorectal cancer (CRC). This article explores the mechanisms by which C. difficile toxins, TcdA and TcdB, contribute to CRC pathogenesis through epithelial barrier disruption, DNA damage, and chronic inflammation via NF-κB and STAT3 activation. Dysbiosis further exacerbates tumorigenesis by altering microbial metabolites. Understanding these interactions highlights potential therapeutic strategies, including toxin-neutralizing antibodies, fecal microbiota transplantation, and anti-inflammatory interventions, to mitigate CRC risk associated with *C. difficile*.

✉ Manouchehr Fadaee, m.fadaee74@yahoo.com | [1]Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran. [2]Infectious and Tropical Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran. [3]Pediatric Health Research Center, Tabriz University of Medical Sciences, Tabriz, Iran. [4]Department of Immunology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran.

**Graphical Abstract**




## 1 *C. difficile* initiate the pathogenesis of CRC

Colorectal cancer (CRC) is one of the leading causes of cancer-related mortality worldwide [1]. The pathogenesis of CRC is complex, involving a combination of genetic, environmental, and lifestyle factors. Common indicators of vulnerability include a diet high in red and processed meats, low fiber intake, weight gain, physical inactivity, smoking, and high drinking habits [2, 3]. As the care of malignant diseases has improved, it has become progressively essential to address the specific demands and challenges faced by cancer survivors in recent decades [4]. Certain intestinal bacteria, especially pathogenic bacteria such as *Clostridium difficile*, can be considered another important factor in the development of CRC [5, 6]. *C. difficile*, traditionally known as the causative agent of antibiotic-associated colitis, has been increasingly recognized as a potential oncogenic agent in recent years [7, 8].

*C. difficile* synthesizes three distinct toxins: toxin A (TcdA), toxin B (TcdB), and a binary toxin known as *C. difficile* transferase (CDT). TcdA and TcdB are chiefly accountable for the pathological properties of *C. difficile*, with TcdB exhibiting greater toxicity than TcdA and serving as the principal contributor to the consequences of *C. difficile* infection (CDI) [9].

The toxins TcdA, TcdB, and CDT are synthesized by *C. difficile* through specific genes in a region of the genome called the toxin transfer gene cluster (tcd). These toxins are mainly encoded by the *tcdA* and *tcdB* genes, which are responsible for the production of the toxins TcdA and TcdB. In addition, the *cdt* gene directs the production of the CDT transferase, which specifically damages the DNA of intestinal epithelial cells [10]. The synthesis process of these toxins is influenced by regulatory systems such as *tcdR* and *tcdC*. *tcdR* acts as an activating factor and stimulates the expression of toxin genes, while *tcdC* acts as an inhibitor and reduces the amount of toxin production [10, 11]. These toxins cause epithelial barrier disruption, cell death, and inflammatory responses, all of which are associated with the pathogenesis of CRC [12]. TcdA, which functions primarily as an enterotoxin, disrupts the integrity of the epithelial barrier by targeting tight junction proteins such as claudins and occludins [13]. This disruption results in increased intestinal permeability, allowing luminal contents such as lipopolysaccharides (LPS), microbial toxins, and mutagenic compounds to penetrate the mucosal and submucosal layers. This process not only creates an inflammatory environment but also exposes intestinal epithelial cells (IECs) to toxic compounds that can damage DNA [14, 15]. TcdA also promotes inflammation through the release of neuropeptides such as Substance P (SP) and Calcitonin gene-related peptide (CGRP) from sensory neurons, thereby amplifying the inflammatory cascade. SP activates intestinal macrophages in the lamina propria, leading to the release of TNF-α and subsequent damage to IECs. In addition, exposure to TcdA enhances the expression of the neurokinin-1 receptor on IECs [9].

TcdB, functioning as a cytotoxin, plays a more direct involvement in cellular destruction. Research indicates that this toxin induces the degradation of DNA molecules by stimulating reactive oxygen species (ROS) generation in IECs [16]. The damage incurred encompasses double-stranded DNA breaks that, if not repaired, result in genetic and chromosomal alterations. These mutations can deactivate tumor suppressor genes or activate oncogenes, both of which facilitate cancer progression [17]. TcdB triggers senescence in enteric glial cells (EGCs) that endure the effects of the toxins , which primarily leads to cell death through either necrosis or apoptosis [18]. TcdB-induced senescence in EGCs primarily results in a permanent arrest of the cell cycle, accompanied by constant injury to DNA, suppression of c-myc, and hypophosphorylation of the phosphorylated retinoblastoma protein (pRb). Senescent cells produced by TcdB experience cell cycle arrest in G0/G1 and G2/M, which is caused by p27 overexpression linked to cyclin-dependent kinase 1 (CDK1) and cyclin B1 downregulation [19]. In addition, TcdB can disrupt the cytoskeleton and induce apoptotic or necrotic cell death by altering signaling proteins such as Rho GTPases, both of which can contribute to inflammation persisting under certain conditions [20].

Another important aspect of the effects of *C. difficile* toxins is their ability to activate inflammatory signaling pathways such as nuclear factor-κB (NF-κB) and signal transducer and activator of transcription 3 (STAT3) [20, 21]. These pathways are accompanied by the extensive production of pro-inflammatory cytokines and chemokines such as interleukin-6 (IL-6), IL-8, and tumor necrosis factor-alpha (TNF-α), which directly create a tumorigenic environment [22]. The chronic inflammation caused by these cytokines not only ensures the survival of damaged cells but also accelerates the proliferation of mutated cells. In addition, persistent activation of STAT3 can prevent programmed cell death and contribute to the survival of precancerous cells by increasing the expression of anti-apoptotic genes such as B-cell lymphoma 2 (Bcl-2) and myeloid cell leukemia 1 (Mcl-1) [23, 24].

In addition to the pathways mentioned, CDIs are often associated with profound changes in the gut microbiota, known as dysbiosis [25, 26]. One of the most impactful effects of dysbiosis is the loss of beneficial microbiota that produces anti-inflammatory metabolites such as short-chain fatty acids (SCFAs, including acetate, propionate, and butyrate) [27]. These metabolites are crucial for maintaining intestinal health, and their reduction can exacerbate the pathological effects of *C. difficile* infection. Dysbiosis promotes the growth of pathogenic bacteria, including *C. difficile*, and aggravates inflammation and epithelial stress. The beneficial effects of SCFAs in mitigating gastrointestinal dysbiosis occur through interactions with IECs and immune cells, which help counterbalance the inflammatory response and support the gut barrier function [28, 29]. Butyrate, acetate and propionate enhance anti-inflammatory procedures through the regulation of the leukocyte and endothelial cell responses, including the synthesis of cytokines (TNF-α, IL-2, IL-6, and IL-10), eicosanoids, and chemokines [such as monocyte chemoattractant protein-1 (MCP-1) and cytokine-induced neutrophil chemoattractant-2 (CINC-2)]. Nonetheless, the pro-inflammatory effects of SCFAs have also been identified in certain situations. The concentrations of SCFAs are associated with changes in the intestinal microbiota and may influence the extent of *C. difficile* infection or suppress bacterial development [30]. So, CDI can also trigger tumorigenesis by these microbial changes.

Additionally, changes in microbial metabolism, including increased production of secondary bile acids, create a favorable environment for the development of CRC [31, 32]. In vivo and in vitro studies also provide further evidence of the role of *C. difficile* in the development of CRC. In mouse $APC^{min+}$ models, it has been shown that tumor growth is accelerated in the presence of chronic CDI. In these studies, $APC^{min+}$ mice infected with mutant TcdB strains that have lost their mitogenic role have been shown to develop fewer tumors, highlighting the role of the toxin in oncogenesis [6, 33]. On the other hand, epidemiological data indicate a higher incidence of CRC in patients with recurrent CDIs [34]. Biopsies of tumors from CRC patients frequently demonstrate the presence of *C. difficile* DNA or toxins, indicating a direct association [35]. Furthermore, an in vivo study showed that the incidence of CRC was significantly higher in patients with C. difficile infection, with a relative risk of approximately 2.7 times higher in these patients [34]. Additionally, another study showed that the rate of *C. difficile* colonization was significantly higher in patients with lymph node metastasis compared to those without lymph node involvement, suggesting that *C. difficile* may also play a role in cancer metastasis [36]. Based on the above, understanding the precise mechanisms of the association between *C. difficile* and CRC could provide opportunities for the development of new therapeutic approaches.

Research has shown that targeting TcdA and TcdB toxins can prevent the development of associated diseases [37]. Currently, antibodies that can neutralize these toxins are being investigated to reduce their negative effects on human health [38]. Additionally, fecal microbiota transplantation (FMT) has also been considered a novel therapeutic approach [39]. This method can reduce the risk of CRC by restoring microbial balance in the intestine. In this way, by enhancing beneficial microbial populations and reducing the activity of pathogenic bacteria, the likelihood of inflammation and cancer-related disorders is reduced [40]. Additionally, the beneficial bacteria that are transferred to the patient during FMT compete for energy sources with *C. difficile* and, by producing organic acids such as lactic acid and butyric acid, create an acidic environment in the intestine that is unfavorable for the growth of *C. difficile*. In addition, beneficial bacteria can play a role in eliminating pathogenic bacteria such as Clostridium difficile by producing proteins called bacteriocins [41]. Furthermore, anti-inflammatory strategies that target specific inflammatory pathways, such as the IL-6/STAT3 pathway, may help reduce tumorigenic effects (42). These inflammatory pathways are involved in cancer development, and their inhibition could lead to reduced growth of CRC. Such approaches may be used as adjunct therapies alongside other existing modalities to increase the overall efficacy of treatments. However, despite providing valuable insights into the potential association between *C. difficile* toxins and CRC, this study has several limitations. Most of the mechanisms discussed are derived from in vitro or animal model studies, and there is a lack of direct evidence in human subjects. Additionally, the complexity of host-microbiota interactions and the influence of environmental or genetic factors were not fully explored. Future studies should aim to validate these findings in clinical settings and human cell models, focusing on the molecular pathways by which *C. difficile* toxins contribute to CRC development. Large-scale epidemiological studies and longitudinal research are also needed to establish a clearer causal relationship.

In conclusion, this study highlights the multifaceted role of *C. difficile* in CRC development, emphasizing the pathogenic effects of its toxins and associated gut microbiota dysbiosis. The evidence underscores the need for targeted interventions to mitigate these effects, such as neutralizing toxin activity, restoring microbial balance, and addressing inflammation-driven tumorigenesis.

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## Declarations

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