Probiotics as an adjuvant for management of gastrointestinal cancers through their anti-inflammatory effects: a mechanistic review

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Abstract

The immune system's role in maintaining the health of the gastrointestinal (GI) system is like a double-edged sword. Simultaneously, it could reduce the risk of pathogen invasion by the inflammatory response. But, if regulated improperly, it could also propagate oncogenic signaling that transfers a normal cell into the malignant counterpart. Thus far, several mechanisms have been proposed, such as the immune system could disturb the GI homeostasis and increase the survival and proliferative capacity of cells leading to the formation of a wide range of malignancies. Among the endless list of these mechanisms, inflammatory responses are currently fascinating research areas, as this response regulation is with the gut microbiota. Given this, the manipulation of microbiota might be a convenient and efficient way to prevent GI cancers. Probiotics could potentially achieve this by overturning the milieu in favor of normal gut homeostasis. In addition to the safety of use of probiotics, along with their potential ability to interact with immune system responses, led these bacteria to be viewed differently from dietary supplements. In the present review, we aimed to look into the mechanisms through which probiotics modulate the immune responses to stimulate anti-inflammatory responses and promote immune surveillance against neoplastic cells.

Keywords: Gastrointestinal cancer, Inflammatory responses, Probiotics, Gut microbiota

1 Introduction

Gastrointestinal (GI) cancer is the most prevalent malignancy worldwide, comprising 25% of all human cancers. The clinical management and prevention of GI cancer are among the main concerns these days [1]. The variety of cancers in this category, the different pathogenic mechanisms, the high mortality rate, and the ineffectiveness of the existing treatments and advances have all made GI cancer one of the leading health problems of the recent century. Colorectal cancer (CRC), gastric cancer (GC), gallbladder cancer, hepatocellular carcinoma (HCC), esophageal cancer, pancreatic cancer, and anal cancer are some of the most well-known malignancies that threaten the life of a considerable number of people globally [2, 3]. Nowadays, we know that genetics and epigenetics aberrancies could convert normal gastrointestinal cells into neoplastic cells. Still, environmental factors such as diet and lifestyle may have a fundamental role in developing the disease [4]. Despite the heterogeneity, the prolonged inflammatory responses in the initial steps of carcinogenesis are common in all GI cancer types. Several clinical investigations have demonstrated that chronic inflammation is the leading risk factor in the pathogenesis of GI cancer. For instance, the tight correlation between hepatitis B, *Helicobacter pylori*, or some autoimmune disorders such as inflammatory bowel diseases (IBD) and the risk of esophageal cancer, HCC, GC, or CRC has been discussed in several reports [5-7]. Moreover, several lines of evidence have suggested the role of inflammatory mediators, such as cyclooxygenase enzymes COX-1 and COX-2 [8], nitric oxide [9], reactive oxygen species (ROS) [10], and inflammatory signaling pathways nuclear factor-κB (NF-κB), c-Jun amino N-terminal protein kinase (JNK), and Janus kinase (JAK)/signal transducer and activator of transcription (STAT) in the pathogenesis of GI cancer [11-14]. Hence, nonsteroidal anti-inflammatory drugs (NSAIDs) have been suggested as possible therapeutic strategies for these malignancies [15]. However, there is a pressing need to understand how inflammatory responses could provide a platform for cancer development in the GI tract.

The correlation between inflammation and cancer has been discovered for a long time. Although inflammatory responses, as an efficient tool of innate immune responses, have protective effects against tissue injury and infection [16], if activated improperly, pro-inflammatory cytokines, the mediators of the inflammatory responses, could aberrantly activate signaling pathways that lead to several human diseases [17]. For a long time, microorganisms such as bacteria, fungi, and even viruses have been suggested to could rebalance the immune responses. For example, it has been

reported that the infection with several types of viruses, in particular human papillomaviruses, could increase the risk of cancer in individuals. Indeed, it seems that through activation of necroptosis –a protective event against viral infections, viruses could increase the cytokine gene expression and thereby trigger carcinogenesis in the targeted tissues [18]. In a study conducted by Haanen et al., it has been suggested that inflammatory responses produced by immune cells such as neutrophils, macrophages, and eosinophils could induce apoptotic cell death in the cells at the inflammatory site [19]. It is now well-established that chronic tissue damage could activate DNA repair mechanisms and thereby increase the risk of cancer development. Another mechanism that could be activated through inflammatory responses is autophagy. When it comes to autophagy and its role in cancer development, the data are controversial. In some cases, it has been reported that the activation of autophagy could act in favor of cancer progression, and in other investigations, autophagy has been claimed to be a tumor-suppressive process. A striking point in the association between autophagy and inflammatory responses is that autophagy could activate inflammatory responses. The best evidence for this finding is in Crohn's disease, where polymorphism in genes encoding Atg2a, Atg4a, Atg4d, death-associated protein, immunity-related GTPase family M protein (IRGM), and ULK-1 have been shown to activate inflammatory responses. Since Crohn's disease could elevate the risk of GI cancer development, it is reasonable to propose that perhaps the cross-talk between inflammation and autophagy system would be a mechanism that leads to carcinogenesis [20].

Albeit the effect of bacteria, viruses, and fungi on human diseases, it should not be forgotten that these microorganisms might have also protective effects. Many GI commensal microorganisms protect the body from either invading pathogens or even some inflammatory-related diseases [21, 22]. The importance of probiotic products in the maintenance of gastrointestinal health is well-established in several studies. It has also been declared that there is a correlation between the type of diet and immune system integrity [23]. For example, the short-term fasting diet in mice has been shown to could induce mucosa atrophy [24, 25]. Even in COVID-19 disease, it has also been claimed that the type of nutrient of the patient influences the progression of the disease [26]. Taken together, these finding suggests that perhaps GI microbiota might be a missing part of the puzzle for the treatment of GI cancer. The present review aims to first take a glance at the role of inflammatory responses in the pathogenesis of GI cancers and then discuss whether probiotics could be considered in the therapeutic regimen of these cancers.

2 Gastrointestinal cancer; a disease with the inflammatory background

The critical role of the immune system in GI cancer development and progression became clinically evident when the results of a study conducted on immune-deficient mice showed the disability of H. pylori in the induction of gastric cancer [27]. It has been claimed that in inflammation conditions, the dysbiosis of H.pylori overgrowths of any strain of the microbiota, and this event causes carcinogenesis [28, 29]. Additionally, similar investigations were also performed in the years before these findings to highlight the importance of immune cells in the progression of GI cancer. In all cases, the lack of information and a thorough understanding of the inflammatory processes restricted the link between these results in the disease's pathogenesis. For example, the results of a study in 1999 revealed the importance of CD4 positive T lymphocytes, and not B lymphocytes, in H.pylori-induced gastric cancer [30]. Similarly, other results also indicated that the CD4/CD3 positive T lymphocytes could increase the development and progression of CRC [31]. Therefore, these findings shed light on the CD4 T lymphocytes' participation and their associated cytokines in the malignant transformation of chronically inflamed tissue.

Several studies have suggested that T helper 2 (Th2) cells and their related cytokines may also be involved in the progression of different GI cancer types. Accordingly, Osawa *et al*. reported that the Th2 cytokines, including interleukin (IL) -4 and IL-5, accelerated tumor development's pace towards a more malignant condition and was associated with a poor clinical outcome [32]. However, activation of Th1 cells in this type of cancer and production of interferon (IFN)-γ and IL-12 prevents the cancer progression by enhancing tumor surveillance and angiogenesis suppression [33-35]. In sporadic CRC, it has also been indicated that while the activation of Th2 develops tumor cell's metastatic potential [36, 37], Th1 stimulation may improve the prognosis of the disease [38]. It seems that IL-4 and IL-13 (produced from Th2) could increase the expression of activation-induced cytidine deaminase (AID), an enzyme with the ability to induce DNA mutations in colonic epithelial cells [39]. Another CD4 T lymphocyte that participates in GI cancer progression is Th17, a lymphocyte responsible for the production of IL-17. Through integrating with the IL-23 signaling pathway, IL-17 enforces immune cells to produce pro-inflammatory cytokines, such as IL-1, IL-6, TNFα, nitric oxide synthase 2, metalloproteases, and chemokines [40-42].

Moreover, it seems that the suppression of CD8 positive cytotoxic T cells and CD4 positive regulatory T cells (Tregs) might impact the progression and development of GI cancer. The previous studies reported that IFN-γ and IL-10-deficient mice are more prone to developing the conditions leading to CRC proliferation than their regular counterparts [43]. No matter which type of T cell would be activated in GI cells, if the pro-inflammatory cytokines are produced in an uncontrolled fashion, they trigger several signaling pathways that alter cell survival and proliferative capacity and cause tumorigenesis in a variety of cancer in the GI tract.

One of the best-studied transcription factors in immune signaling is NF-κB, a rapid-acting nuclear transcription factor that can link inflammatory responses to oncogenic processes [43]. The unique characteristic of NF-κB allows this transcription factor to be the first responder to extra- and intracellular stimuli. When pro-inflammatory cytokines (IL-1, IL-6, and TNFα) facilitate NF-κB to enter the nucleus, this transcription factor binds to the promoter of a wide range of genes [44]. It increases the expression of anti-apoptotic genes (*Bcl-2* and *IAP* family), nitric oxide synthesis (iNOS), the catalytic subunit of telomerase (hTERT), COX-1/-2, multi-drug resistant proteins, and different metalloproteinases; each of these targets is notorious for its role in various stages of tumorigenesis. The up-regulation in even one of them is sufficient to transfer a normal cell to a malignant counterpart. Through up-regulating anti-apoptotic target genes and hTERT, NF-κB prolongs the survival of malignant cells [6]. By increasing the expression of iNOS, this transcription factor elevates the intracellular amount of free radicals, which could induce mutations in the genome or impair the DNA repair system. Also, by regulating MDR proteins and MMPs, NF-κB confers upon cancer cells the ability to resist chemotherapeutic agents and invade them, respectively [45, 46]. The footprint of NF-κB is evident in most types of GI malignancies, including hepatocellular carcinoma and colorectal cancer suggesting its significant role.

3 What initiates inflammatory responses in GI cancer? The role of microbiota in disease progression

For a long time, bacterial and viral infections were considered the primary drivers that trigger the immune response in favor of GI cancer progression. It has been claimed that bacteria are responsible for stimulating innate immunity to initiate intestinal inflammation and subsequently activate adaptive immunity to establish and maintain chronic inflammation in the gut environment [47].

When the attention is drawn to GI microbiota, a new chapter opened in the GI cancer pathogenesis, focusing on the intrinsic invaders. In general terms, GI microbiota comprising more than hundreds of bacteria, viral and fungal species, construct a gut barrier against pathogen invaders [48]. Moreover, by producing the necessary cytokines, these microorganisms modulate the innate and adaptive immunity in the GI tract preventing the formation of any malignancies [49, 50]. The results of in-depth investigations showed a significant difference between the microbiota of healthy individuals and those who have GI cancer. For example, while the most fecal bacterial population of healthy people includes *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, and *Actinobacteria* [51], stool sample of patients with CRC contains *Escherichia* and *Fusobacterium* bacteria [52]. A substantial body of literature has provided evidence for gut microbiota's role in the pathogenesis of GI cancers, including HCC [53]. Although the liver does not have a microbiome, the portal vein could bridge intestinal microbiota and the liver [54]. It seems that the disruption of the balanced population of microbiota, in the primary stage, impairs the intestinal barrier and stimulates inflammatory responses. The aberrant production of cytokines, chemokines, and inflammatory mediators could activate the oncogenic signaling pathways, such as phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt), mitogen-activated protein kinase (MAPK), and NF-κB, which leads to DNA damage through excessive production of free radicals like ROS and NOS [55, 56]. More than anything, these findings shed light on the importance of microbiota in the development of GI-related malignancies. Given these results, again, one question that has occupied researchers' minds is whether manipulation of gut microbiota using probiotics could change the GI microenvironment in favor of cancer prevention and progression.

4 Probiotics; a solution to prevent GI cancer or a magic wand for the treatment of tumor

According to the Food and Agriculture Organization of the United Nations and World Health Organization Expert Consultation, probiotics are a group of living bacteria that initially prevent the housing of pathogenic microorganisms in the GI tract [57, 58]. When it comes to probiotics and the benefits of taking them, *Lactic acid bacteria* (LAB) and *Bifidobacteria* are the first type of bacteria that comes to mind; however, some other yeasts and bacilli may be considered probiotics as well. Next to the protective roles against pathogenic microorganisms, it now becomes clear that probiotics could also trigger the immune surveillance against any malignancies through modulating anti-inflammatory, immune responses or interacting with cytotoxic lymphocytes Treg

[54, 57, 59-62]. There is a general point of view that probiotic bacteria could be used as a tool to modulate the microbiota and potentiate gut barrier function, thereby preventing cancer development and progression [63, 64].

4.1 Probiotics and their role in the prevention of GI cancer

In probiotics, lactic bacteria, originally belonging to the *Lactobacillus* and *Bifidobacterium* families, have attracted tremendous attention [65]. Although several microorganisms have been considered probiotics, a prominent identifying feature shared among all or most is that they are Gram-positive [66]. In recent years, the administration of probiotic bacteria to maintain GI tract health or prevent and treat GI-related malignancies has received significant attention [67]. For instance, the beneficial impact of feeding milk fermented with a mixture of *Lactobacillus casei* (*L. casei*) and *L. acidophilus* in the induction of apoptotic cell death of tumor cells has been reported in a study conducted by Lee *et al*. [68] and Baldwin *et al*. [69]. Similarly, the anti-proliferative activity of the *L. rhamnosus* GG strain was also reported in gastric cancer patients [70, 71].

Moreover, in another study, it has also been indicated that exposing colon cancer cells to a probiotic product *Bifidobacterium adolescentis* SPM0212, exerts a marked reduction in cell proliferation, suggesting the antitumoral potential of probiotic products [72]. The list of probiotics that could exert anti-cancer effects in GI cancer is endless, and the brief information about their antitumor activity is summarized in Table 1. Although probiotics are an excellent candidate for cancer therapy, their action mechanism is still deficient and ambiguous.

Thus far, several mechanisms have been proposed to explain the anti-cancer properties of probiotics. Normalizing GI microbiota, improving gut barriers, and eliminating oncogenic pathogens are some of the most suggested mechanisms. However, in the long list of these mechanisms, propagating the anti-inflammatory responses seems to be the primary weapon through which probiotics halt the progression of neoplastic cells. In the following part of this review, some of the most important mechanisms through which probiotics might exert their antitumor activities will be discussed.

Table 1. The list of probiotic bacteria and their effects on the management and treatment of GI cancers.

Probiotics	Type of intervention	Description	Ref
Colorectal cancer (CRC)			

*L. rhamnosus (*LC705) & Propionibacterium freudenreichii ssp shermanii JS (PJS)	prevention	Reduced the expression of beta-glucosidase, a vital enzyme that participated in carcinogenesis in healthy individuals.	[73]
RS *vs* BF lactis	prevention	Changed the microbiota population in favor of cancer prevention.	[74]
L. gasseri (LG21)	prevention	CRC patients and healthy counterparts were subjected to probiotics. The probiotic ingestion shifted the ratio of Lactobacillus to Clostridium perfringens in favor of lactobacillus. While there was a significant reduction in fecal pH and fecal putrefaction products, the synthesis rate of short-chain fatty acid isobutyric acid was increased.	[75]
L. acidophilus, L. plantarum, B. lactis BB, S. boulardii	prevention	CRC patients received probiotics after surgery. Probiotics decreased the risk of post-surgery complications. Also, it reduced the expression of TNF and IL-6 in CRC patients.	[76]
L. rhamnosus GG	Supportive Effect	Had supportive effects with chemotherapeutics and reduced the side effects of chemotherapeutic agents, such as diarrhea, abdominal pain, and fewer.	[77]
Lactobacillus rhamnosus GG	Therapeutic Effect	Induced apoptotic cell death in Caco–2 cells through decreasing IL-8.	[78]
Gastric cancer (GC)			
L. casei *ssp* and L. casei *DG*	Supportive Effect	Although probiotics could not eradicate H.pylori in GC patients, they could alleviate the side effects of antibiotics-based treatment in the patients.	[79]
L. acidophilus *La5 and B.* lactis *Bb12*	Therapeutic Effect	Not only reduced the number of H.pylori in GC patients but also decreased the side effects.	[80]
L. casei	Therapeutic Effect	As compared to the patients who received antibiotics, those subjected to both anti-biotics and L.casei had a lower number of H.pylori.	[81]
L. acidophilus *La5 and B.* lactis *Bb12*	Therapeutic Effect	Enhanced the Effect of antibiotics to eliminate H. pylori in patients.	[82]

L. reuteri	Therapeutic Effect	Enhanced the Effect of antibiotics to eliminate H. pylori in patients.	[83]
Other GI cancer			
L. casei	Therapeutic Effect	Induced anti-cancer effects in 5-FU-resistant pancreatic cancer cells in vitro and in vivo in a mouse xenograft model.	[84]
Lactobacillus paracasei GMNL-133 and *Lactobacillus reuteri GMNL-89*	Adjuvant Effect	Induced synergistic Effect on gemcitabine-induced anti-proliferative Effect in a murine pancreatic model.	[85]
Lactobacillus reuteri and Lactobacillus paracasei	Therapeutic Effect	Inhibited Kras-induced pancreatic cancer development and ameliorated P. gingivalis-induced cancer in mice.	[86]
Lactobacillus acidophilus and Bifidobacterium longum	Therapeutic Effect	Induced anti-proliferative effects on bladder cancer cells through COX2 downregulation.	[87]
Bifidobacterium bifidioum	Therapeutic Effect	Prevent the progression of metastasis of hepatocarcinoma (HCC) in mice by altering microRNA expression profiles.	[88]

4.2 Probiotics in the clinical trials

Given the promising effects of probiotics in the preclinical investigations, these bacterial products also find their way into clinical trials. For example, in one study, cancer patients were treated with VSL#3, which is a mixture of several Lactobacilli strains (Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus casei, and Lactobacillus bulgaricus), Bifidum strains (Bifidobacterium breve, Bifidobacterium longum, Bifidobacterium infantis), and Streptococcus thermophiles. This study aimed to evaluate the protective effects of probiotics against the gastrointestinal side effects of the chemotherapeutic drugs (NCT03704727). In another study for colorectal neoplasm, patients were treated with probiotics after colectomy and the results suggested that probiotics could increase the survival of the patients (NCT01479907). The effect of probiotic regimen on the expression of IL10, IL1β, IL23α, TNF, IL12β, INFγ, IL17α was also evaluated in another clinical trial. They treated colorectal cancer patients with a dietary supplement containing Saccharomyces boulardii for seven days and then the expression of the aforementioned cytokines was evaluated (NCT01895530). In the phase 4 clinical trial, the impact of Saccharomyces boulardii was evaluated on the expression profile of several pro-inflammatory cytokines in colorectal cancer

(NCT01609660). Another clinical trial also indicated that Saccharomyces boulardii could maintain the integrity of the epithelial barrier and prevent the induction of infection after colorectal resections in colon cancer patients (NCT01609660). Dietary Supplement of Lactobacillus Rhamnosus also showed to could prevent chemotherapy-induced diarrhea in colorectal cancer patients (NCT00197873). In patients with rectal cancer, it became evident that probiotic bacteria could reduce the radiation-induced inflammation in the gastrointestinal mucosa (NCT03420443). Apart from these trials, other investigations evaluated the safety and efficacy of probiotics in the treatment strategies of GI cancers. For example, a phase II/III clinical trial in colorectal cancer is still recruiting patients to evaluate the benefits of probiotics as adjuvant therapy in colorectal cancer (NCT03742596). It should be noted that some of these studies in this area were also terminated or withdrawn due to slow recruitment (NCT02351089, NCT01473290, NCT04131803). Taken together, these investigations showed that probiotics might need a long way to go to find their place in the treatment of GI cancers.

4.3 **The antitumor activity of probiotics; mechanism of action**

4.3.1 *Probiotics and normalizing gut microbiota*

It is well-established that some of the intestinal microorganisms have a critical role in developing GI-related cancers via producing toxic and genotoxic bacterial metabolites [89]. These poisonous metabolites could either interfere with different intracellular signaling pathways, leading to uncontrolled cell survival and proliferation or be incorporated into the genome to induce mutations in essential genes [90]. Given these, it seems that probiotics could be a suitable candidate for recruiting beneficial bacteria into the GI tract to maintain the balance of microbiota by diminishing the population of detrimental bacteria [91]. Firstly, by having a competitive behavior with pathogenic microorganisms, the probiotic bacteria could conveniently adhere to the host epithelial cells and reduce the remaining sites to reproduce pathogenic bacteria. Secondly, many probiotic bacteria produce some antimicrobial compounds, particularly bacteriocins and antibiotics, that could reduce the population of a wide range of pathogenic bacteria [92]. Moreover, the settlement of probiotics in the GI tract ultimately improves the integrity of the gut barrier, diminishing the risk of pathogen invasion.

4.3.2 *Probiotics and production of antitumor compounds*

Aside from preventing the colonization of unfavorable bacteria, probiotic bacteria could also produce compounds with an antitumor activity that eventually diminish the survival and proliferative capacity of tumor cells [72]. *L. casei* could induce Tumor necrosis factor Related Apoptosis-Inducing Ligand (TRAIL)-dependent apoptotic cell death in both murine (CT26) and human (HT-29) colon carcinoma cells lines [93]. Moreover, other study results indicated that exposure of HT-29 cells to 10^9 CFU/mL *L. casei* for 24 h resulted in a decrease in *survivin* expression, as an essential member of the inhibitor of apoptosis (IAP) family [94]. The cell-bound exopolysaccharides (cb-EPS) isolated from *Lactobacillus acidophilus* are also shown to have an antitumor effect on colon cancer. In the study conducted by Kim *et al*., it has been reported that when colon cancer-derived HT-29 cells were subjected to cb-EPS, the survival capacity of the cells was significantly reduced as a result of activation of autophagy and apoptotic cell death. It is worth mentioning that stimulation of autophagy in tumor cells could rapidly digest the vital proteins of tumor cells affecting its survival. Furthermore, it has been indicated that the secreted cb-EPS significantly enhanced the expression of Beclin-1, a necessary protein for the formation of the autophagosome. In the meantime, these exopolysaccharides could increase the intracellular ratio of death promoter (*Bax*) to death repressor (*Bcl2*) in HT-29 cells [95]. The integration with mitochondrial function to induce apoptotic cell death in cancer cells has been reported for *Lactobacillus rhamnosus* and *Bifidobacterium lactis* [96]. Moreover, *Propionibacterium freudenreichii* also caused apoptotic cell death in both colon and gastric cancer cell lines by producing a short-chain fatty acid to culture media [97]. The success of probiotics in eliminating the survival of malignant cells is not restricted to these observations. There is some evidence suggesting that administering these bacteria could also enhance the chemotherapeutic agents' therapeutic value. The fatty acid secreted from *Propionibacterium freudenreichii* potentiated camptothecin's anti-cancer effect, a drug used in gastric cancer treatment [98]. Similarly, in another study, it has been reported that the compounds produced from *Lactobacillus acidophilus* and *L. casei* could increase the sensitivity of colorectal carcinoma-derived LS513 cells to 5-fluorouracil [69].

4.3.3 *Probiotics and modulating immune system*

By interacting with different immune system cells such as dendritic cells, T, and B lymphocytes, probiotic bacteria could improve the immune responses of the GI tract to more effectively remove pathogenic bacteria or inhibit the growth of malignant cells [99]. These bacteria's effects on the immune system's modulation could be mediated by producing anti-inflammatory substances, anti-oxidant compounds, and regulating the cellular adaptive immune system [100, 101].

4.3.3.1 Probiotics regulate anti-inflammatory responses by interacting with T helper cells

When it comes to the anti-cancer effects of probiotics in GI cancer, the first mechanism of action that comes to mind is modulating inflammatory responses. The results extracted from previous studies suggested that probiotics could suppress inflammatory responses by directly inhibiting pro-inflammatory cytokine production or interacting with signaling pathways such as NF-κB. For instance, while *L. rhamnosus* GG can counteract IBD-mediated colon cancer progression through interfering with NF-κB signaling axis [102], *viable* Escherichia coli *Nissle 1917 (EcN),* and *heat-inactivated VSL#3* could reduce the survival of mice model of HCC through blocking IL-17-mediated inflammatory responses [94]. We will now discuss various ways in which probiotic bacteria could modulate immune responses.

One way probiotic bacteria prevent the colonization of pathogens in the GI tract is by altering the intracellular ratio of pro-inflammatory to anti-inflammatory cytokines [103]. As mentioned earlier, while Th2 and its related cytokines (IL-4 and IL-13) have an essential role in the induction of GI cancers, Th1 has anti-cancer effects. It has been suggested that many probiotic bacteria such as *Lactobacillus* enforce Th1 cells to produce IFN-γ and IL-12, two cytokines that stimulate effector cytotoxic T (CD8 positive) cells [104]. IL-12 also has an immune-stimulatory effect on natural killer (NK) cells, an active immune cell during malignancies. Activated cytotoxic T cells and NK cells then go hand in hand to eliminate the malignant cells by interacting with them and reducing their proliferative capacity [105]. Rather than activating cytotoxic T cells and NK cells, the IFN-γ produced by probiotics may also stimulate immunoglobulin class switching in B lymphocytes. It has been shown that *Lactobacillus acidophilus, L. casei*, and *Bifidobacterium* spp not only increase the proliferative capacity of B lymphocytes through elevating the blood level of IFN-γ but also potentiate the production of immunoglobulin A (IgA) [106]. IgA, as the most abundant antibody found at the mucosal surface in the form of secretory IgA (sIgA), sustains the GI tract's health by maintaining the population of non-invasive commensal bacteria or by neutralizing the invasive

pathogens. The sIgA recruits innate immune cells to intercept toxins and prevent the adhesion of pathogens to the intestinal epithelial cells (IECs). One of the unique characteristics of sIgA in protecting the intestinal lumen is that this antibody exerts its effect without activating the complement cascade, thereby hampering inflammatory damage to the epithelial barrier [107]. Given these, it seems that the elevation of the sIgA secretion by probiotics is associated with the favor of GI tract health and is the mechanism through which these bacteria could counteract the detrimental effects of inflammatory responses. The evidence supporting that probiotic bacteria may increase the production of sIgA, which was also evident in that *L. casei* and *Bifidobacterium animalis,* in murine and human models, respectively [108, 109].

Aside from Th1, some probiotic bacteria strains also interact with Th2 cells to suppress the secretion of inflammatory cytokines. In some studies, it has been reported that *Lactobacillus* species interact with Th2 cells, preventing the secretion of IL-4 and IL-5 from these cells, thereby blocking inflammatory responses [104]. It is well-established that IL-5 is known for its role in regulating innate and acquired immune responses. By interacting with the IL-5 receptor (IL-5R) expressed on eosinophils and mast cells, this interleukin increases inflammatory mediators' release and develops chronic inflammation [110, 111]. Additionally, the over-expression of IL-5 is associated with enhanced cell proliferation and survival. Given these multi-dimensional functions, suppression of IL-5 production by probiotics would be promising for the prevention and treatment of GI cancers. Fig. 1 has brief information about the mechanisms through which probiotics might reduce GI cancer risk.

4.3.4 Probiotics regulate anti-inflammatory responses by producing short-chain fatty acids

So far, it became evident that probiotic bacteria could interact with various immune cells and modulate multiple cytokines' expression profiles. This ability could be regulated through several mechanisms. The metabolites produced by probiotics might change the intracellular events so that the expression of some cytokines would be inhibited or stimulated [112, 113]. The results of in-depth investigations found that short-chain fatty acids might hold a significant share in the immunomodulatory functions of probiotics. Through binding to short fatty acid receptors (SFARs), which mostly belong to the G protein-coupled receptor (GPCR) family, these metabolites activate multiple signaling pathways, leading to anti-inflammatory cytokines production. Free fatty acid receptor 3 FFAR3 or (GPR41) and niacin receptor 1 (GPR109A) are two crucial SFARs that are expressed on the surface of intestinal epithelial cells, immune cells,

and adipocytes [114]. The butyric and acetic acid produced by *Lactobacillus fermentum NCIMB* 5221 is one of the best examples showing the impact of probiotics metabolites on the cytokine expression profile [115]. Once binding to GPR109A [116], the produced butyric acid barricades macrophages to secret pro-inflammatory cytokines (IL-6 and IL-12) in response to lipopolysaccharide (LPS) [117]. Furthermore, the metabolite produced by *B. breve* and *Streptococcus thermophiles* was also reported to suppress LPS-induced TNFα production through inhibiting the NF-κB signaling axis [118] (Fig.2).

L. acidophilus counteracts the inflammatory responses induced by H. pylori infection in gastric epithelial cells through generating conjugated linoleic acids (CLA). This fatty acid could prevent the dissociation of inhibitory IkBa from the NF-κB complex [119]. Another fatty acid that could suppress the NF-κB signaling axis activity in immune cells is propionic acid. This fatty acid produced by various probiotic bacteria interacts with cell surface receptor GPR43 (91) to provoke apoptotic cell death in tumor cells. It might also inhibit the production of pro-inflammatory cytokines from different immune cells [120].

c) Probiotics regulates anti-inflammatory responses through stimulating PPARγ and its related signaling

SFARs are not the only receptors on either epithelial or immune cells capable of interacting with short-chain fatty acids produced by probiotics. Another receptor that can transfer the anti-inflammatory message of probiotics to the nucleus is the peroxisome proliferator-activated receptor-gamma (PPARγ). This nuclear receptor has a tight association with the expression of a wide range of genes [121, 122]. Once the interaction between PPARγ and probiotic metabolites is identified, a new chapter has opened on the beneficial impact of these bacteria in managing GI cancers. One of the most critical roles of PPARγ in the GI tract is the maintenance of microbial homeostasis through regulating glucose and lipid metabolism. In the lack of butyrate (one of the main ligands of PPARγ) and thereby inactivation of PPARγ, the intestinal lumen conditions become anaerobic. As a result, anaerobic bacteria, such as *Proteobacteria*, could grow in it. The growth of *Proteobacteria* results in the activation of macrophages and the production of pro-inflammatory cytokines. Given this, the produced butyrate by probiotics could overturn all of these conditions in favor of anti-inflammatory responses [123, 124]. Besides, the anti-inflammatory effects of *B. thetaiotaomicron* in GI cells are due to the activation of PPARγ [125]. Some strains of probiotics also stimulate the PPARγ signaling pathway by producing CLA. For example, *VSL#3*

has been reported to could successfully prevent colitis-induced CRC by CLA-dependent activation of PPARγ in macrophages [126]. The same results have also been reported for other probiotic bacteria, such as *Lactobacillus acidophilus, L. bulgaricus, L. casei, L. plantarum, Bifidobacterium breve, B. infantis, B. longum,* and *Streptococcus thermophiles*, which could remarkably increase the activation of PPARγ in CRC derived HT-29 and Caco-2 cells, suggestive of the therapeutic value of probiotics in controlling inflammation and preventing colorectal cancer [127]. The anti-inflammatory role of activated PPARγ in immune cells or even in GI epithelial cells could be attributed to the regulatory impact of this nuclear receptor on the expression level of COX-2, one of the essential enzymes involved in GI carcinogenesis [128].

The activation of PPARγ in GI epithelial has another advantage. The results of previous investigations suggested that the activation of PPARγ in GI epithelial cells is also associated with Wnt/β-catenin signaling axis inhibition. The Wnt signaling pathway is located downstream of several signaling cascades. It is notorious for its significant participation in malignant progression or in endowing cancer cells with the ability to proliferate and invade other organs [128]. Moreover, multiple lines of evidence linked the activated PPARγ to induce apoptotic cell death, regulation of autophagy, and NF-κB signaling pathway inhibition. Putting all of these antitumor effects aside; one of the essential advantages that activation of PPARγ could bring for GI cells is the activation of a tumor suppressor protein PTEN [129]. PTEN is the primary regulator of the PI3K/Akt signaling axis, an oncogenic signaling pathway participating in the pathogenesis of gastric and colon cancer [130, 131]. Since the main focus of the present review is on the immune-modulatory role of probiotics in GI cancers, it should be noted that probiotic bacteria could exert their anti-inflammatory effects on GI cancer cells through the PPARγ-mediated up-regulation of PTEN [132, 133]. Besides, a study conducted by Maleki-Kakelar *et al.* has indicated that *Lactobacillus plantarum* prevents H. pylori-induced gastric cancer through PTEN-mediated suppression of toll-like receptor (TLR)-4 [134]. Collectively, *Lactobacillus acidophilus and Lactobacillus rhamnosus GG* were also suggested to reduce the activity of NF-κB, COX-2, and β-catenin through PPARγ-mediated up-regulation of PTEN [135] (Fig. 2).

4.4 The tolerogenic impact of probiotics in immune responses; an Achilles heel of probiotics

Although numerous studies, thus far, focus on the beneficial effect of probiotics in the prevention and management of GI cancers, the influence of these beneficial bacteria is not always hopeful.

Several studies show that in some cases, probiotic bacteria might increase the expression of some immune-suppressive cytokines such as IL-10 and transforming growth factor (TGF)-β. Accordingly, *L. casei*, *L. salivarius,* and *B. breve* have been reported to increase the expression of IL-10 in the GI tract [136-138]. Moreover, the VSL#3 probiotic mix, a cocktail comprising from different *Bifidobacteria,* could enforce mature dendritic cells to produce IL-10 [139] and increase the mucosal Treg [137, 140]. These studies suggested that the production of these cytokines might help restrict the ability of Th cells to produce pro-inflammatory cytokines. Despite the favorable effects of IL-10 in controlling the inflammatory responses and ameliorating some GI tract inflammatory diseases, in particular IBD, it could induce immune tolerance against tumors in the GI tract. It is well-established that IL-10 and TGF-β are two important immune-suppressive cytokines whose activities are attributed to the function of a group of T cells known as Tregs. Besides, Tregs found a fundamental role in treating cancers, as these groups of immune cells could conveniently subvert beneficial antitumor immunity of tumor-infiltrating lymphocytes (TILs) [141]. The results of previous studies have shown that when TGF-β binds to its receptor expressed on $CD4^{+}CD25^{-}$ non-Treg cells, it converts these cells into $CD4^{+}CD25^{+}$ Tregs by MAPK-dependent increased expression of Foxp3. The induced Tregs then release IL-10 in the tumor microenvironment, which in turn induces intratumoral T cell exhaustion by increasing the expression of inhibitory receptors such as BLIMP1 [141]. In other words, Tregs' excessive activation and its associated cytokines (IL-10 and TGF-β) could potentially induce immune tolerance against tumors. This mechanism could bring an opportunity for malignant cells to grow and invade other organs.

Beyond the obvious conclusion drawn about the tolerogenic property of probiotics, it should be kept in mind that there are a considerable number of studies evaluating the therapeutic effects of different *Lactobacillus* spp in various inflammatory diseases of the GI tract. These studies suggested that administrations of probiotics might be advantageous for preventing tumor development, followed by such conditions by producing IL-10 [142]. Nevertheless, it seems that "this is a matter of timing. Perhaps probiotic-induced IL-10/TGF-β production would be beneficial if it was administered at the primary stage of inflammation and not during tumor development. In this vein, it could be the Yin-Yang function of probiotics in the treatment of GI tract-related cancers.

5 Conclusion and future prospective

This literature review will fuel new interest in applying probiotics in the management and GI cancer treatment. All these studies show that by modulating several mechanisms, probiotic bacteria could alter the condition of the neoplastic cells' ability to survive in the gut environment. However, in some cases, the tolerogenic property of probiotics might also cause some complications for the treatment of cancer. This finding suggests that probiotics might be a promising strategy for preventing GI cancer, especially in cases that an inflammatory condition such as autoimmune diseases may exist. Or, perhaps the probiotics could be administrated to GI cancer patients in the presence of immune checkpoint blockades (ICBs), a group of immune-therapeutic drugs that can block the activation of CTLA-4, PD-1, and PD-L1 on regulatory T lymphocytes. The success of different ICBs in the induction of cell death in tumor cells and the prevention of tumor recurrence, led to the administration of these drugs for a variety of cancer patients to the extent that so far, ipilimumab (CTLA-4 inhibitor), Nivolumab, Pembrolizumab (PD-1 inhibitor) received their FDA approval for the treatment of melanoma, lung cancer, kidney cancer, bladder cancer, head and neck cancers. It seems that the combination of probiotic bacteria and ICBs could bring promising therapeutic outcomes for GI cancer patients. However, further experiments must validate the safety and efficacy of this treatment strategy in GI cancer patients. Overall, beyond the obvious conclusion which can be drawn, it seems that these old friends, which have been living commensally with us for a long time, may be able to help human survival once again and would be the solution to fight one of the deadliest diseases of the century.

Conflict of Interests

The authors declare no conflict of interest related to the publication of this article.

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7 Figure legends:

Fig. 1. Schematic representation describing the impact of probiotics on T lymphocytes. Probiotics could inhibit IL-4 and IL-5 by suppressing the activity of T helper (Th)-2 cells. On the other hand, the stimulatory effects of probiotics on Th1 and natural killer (NK) cells could provoke the acquired arm of the immune system to prevent the survival of tumor cells. The produced IFN-γ induced IgA isotype switching in B cells, and elevate the level of secretory IgA in the gastrointestinal tract and stimulate CD8 positive T lymphocyte to counteract with tumor cells. Probiotics also increased the production of IL-12, a key cytokine for NK cell proliferation and activation. The activated NK cells also attack tumor cells and reduce their survival.

Fig. 2. The anti-tumor activity of probiotics is mediated through the production of short-chain acid fatty acids. When produced short-chain-fatty acids interact with short-chain fatty acid receptors (SCFARs) expressed on immune cells, they could recruit G proteins to activate Iκ-B, a well-known inhibitor NF-κB. Once NF-κB is suppressed within the immune cells, the cells' ability to produce pro-inflammatory cytokines such as IL-6, IL-1, and TNFα would be paralyzed. Apart from immune cells, short-chain fatty acids also interact with PPARγ expressed on the nucleus of gastrointestinal cells. Activated PPARγ, on the one hand, inhibits the expression of COX2. This enzyme participates in inflammatory responses, and on the other hand, it activates PTEN, a well-known inhibitor of the PI3K/Akt signaling axis. PPARγ also has a suppressive impact on the Wnt signaling axis. As a result of PI3K/Akt and Wnt signaling axis abrogation, the GI cells could no longer proliferate or survive uncontrollably.