

Antitumor mechanisms of bifidobacteria (Review)

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Abstract. Cancer remains one of the most common causes of mortality globally. Chemotherapy, one of the major treatment strategies for cancer, primarily functions by targeting the cancer cells and affecting them physiologically, but also affects normal cells, which is a major concern at present. Therefore, adverse effects of chemotherapy drugs, including myelosuppression and liver and kidney damage, are of concern. Now, microbial products have attracted attention in cancer treatment research. Notably, carcinogenesis is considered to be associated with microbial dysbiosis, particularly the positive antitumor effects of bifidobacteria. Although there remains a substantial amount to be understood about the regulation of bifidobacteria, bifidobacteria remain an attractive and novel source of cancer therapeutics. The present review focuses on introducing the latest information on the antitumor effects of bifidobacteria and to propose future strategies for using bifidobacteria in the development of cancer therapeutics.

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1. Introduction

Bifidobacterial species are part of the normal human microflora, and exert probiotic effects in humans (1). Previously, several studies reported that bifidobacteria exhibit certain antitumor effects on the development of cancer (2-4). It may work through the mechanisms of fermentation (5), biotransformation (6) and strengthening the intestinal barrier (7), and may potentially function as a treatment method (Fig. 1). For example, *Bifidobacterium breve*, *Bifidobacterium bifidum* and *Bifidobacterium longum* strains isolated from breastfed infants were associated with the fermentation of caprine milk oligosaccharides (8). Owing to the expression of β -galactosidases (9), they were able to utilize 3'- and 6'-sialyl-lactose as growth substrates when they were included as the only carbon source (8).

Apart from their fermentation function, bifidobacteria may also serve an important function in bioconversion, as they may convert ginsenoside into a deglycosylated form under controlled conditions (10). Furthermore, they affect the composition of gut microbiota; for example, *B. breve* UCC2003 exhibited growth properties in a mucin-based medium only in the presence of *B. bifidum* PRL2010, which was demonstrated to metabolize mucin (11). Additionally, the production of specific molecules secreted by *B. bifidum* prevented adhesion attachment and invasion by food-borne pathogens (12). Lactic acid bacteria, including bifidobacteria, were revealed to exert chemopreventative effects on colon, bladder, liver, breast and gastric cancer types (13). The present review discusses the mechanisms involved in the antitumor effect of bifidobacteria.

2. Biotransformation function

Biotransformation is among one of the mechanisms by which bifidobacteria exhibit antitumor effects. Essentially, biotransformation function is fulfilled by the conversion of a compound into a usable energy source via a biological process. High numbers of *Bifidobacterium* may be involved in enterolactone production, which has antitumor effects (14). *Bifidobacterium* spp. may ferment polyunsaturated fatty acid (linoleic acid) into pectic oligosaccharides (POS), which may delay the development of leukemia and associated cachexia in mice, and, consequently, POS may increase the amount of *Bacteroides* spp. (15).

Bifidobacteria may also metabolize certain drugs into therapeutically active compounds against a tumor *in vitro* (6),

including lapachol and 5-fluorocytosine. Oliveira Silva *et al.* (16) performed experiments using two probiotic strains from the human gut: *Bifidobacterium* spp. and *Lactobacillus acidophilus*. Each of them was incubated with lapachol, an anticancer drug, for 12 h in an anaerobic atmosphere at 37°C (16). The culture broths were extracted twice using ethyl acetate, prior to analysis of the chemical profiles of all crude extracts by an injection of 20 μ l of all crude extracts (tests and controls) from the culture broths at 1 mg·ml⁻¹ using reversed-phase high-performance liquid chromatography. A total of 10⁶ colony-forming units (CFU)/ml of bifidobacteria was able to convert lapachol into an active compound against breast cancer cell at 37°C in an anaerobic atmosphere (16).

Additionally, they may convert nontoxic prodrugs into therapeutically active compounds based on the expression of certain enzymes, including cytosine deaminase (17). This enzyme has the ability to convert the nontoxic prodrug 5-fluorocytosine into 5-fluorouracil, which may inhibit the proliferation of carcinoma cells (18). An antitumor drug named ginsenoside Rb1 may be metabolized into the bioactive compound K when incubated with *Bifidobacterium* spp. (19). Furthermore, the gut microbiota was additionally analyzed in people with differing levels of ginsenoside Rb1 degradation into compound K (6). A total of 5 samples with fecal activity potentially metabolizing ginsenoside Rb1 to compound K (FPG) and 5 samples with fecal activity not metabolizing ginsenoside Rb1 to compound K (FNG) were selected from a pool of 100 patients, and the fecal microbiota were analyzed using 16S ribosomal RNA gene pyrosequencing. It was revealed that the population levels of *Bifidobacterium* were substantially increased in the FPG group (6). Additionally, lactic fermentation with *Bifidobacterium longum* subsp. *infantis* may enhance the antitumor cell proliferation effect of soymilk against HT-29 and Caco-2 colorectal cancer (CRC) cell lines (5).

Bifidobacterium additionally serves a notable function in drug metabolism *in vivo*, as the consumption of a mixture of goat probiotics, including bifidobacteria, was associated with a decrease in fecal putrescine (a cancer biomarker) and the reduction of mutagen fecal concentration, including benzo(α)pyrene [B(α)P], which is involved in the onset of CRC (20). Bifidobacteria demonstrated a strong anti-mutagenic effect on B(α)P (21), indicating its function in the prevention of CRC. In addition, the expression and secretion of signal peptides improved the therapeutic effects of bifidobacteria (22). *B. longum* subsp. *infantis* exhibited sustainable antitumor growth activity via downregulating peroxiredoxin-1 through the nuclear factor- κ B signaling pathway in a rodent bladder cancer model (23). A previous study by Xiao *et al.* (24) used *B. longum* subsp. *infantis* thymidine kinase (TK) to treat tumor-bearing nude mice, and revealed that *B. longum* subsp. *infantis* TK resulted in substantially effective antitumor activity and a stronger apoptotic response through intrinsic and extrinsic apoptotic pathways compared with normal saline, *B. longum* subsp. *infantis* or *B. longum* subsp. *infantis*/pGEX-1 λ T treatment. A previous observation noted that the dietary administration of lyophilized cultures of *B. longum* suppressed colon and mammary carcinogenesis in laboratory animal models (25). Furthermore, aberrant crypt foci (ACF) may be recognized as early neoplastic lesions, particularly large ACF (26); and ACF induced by

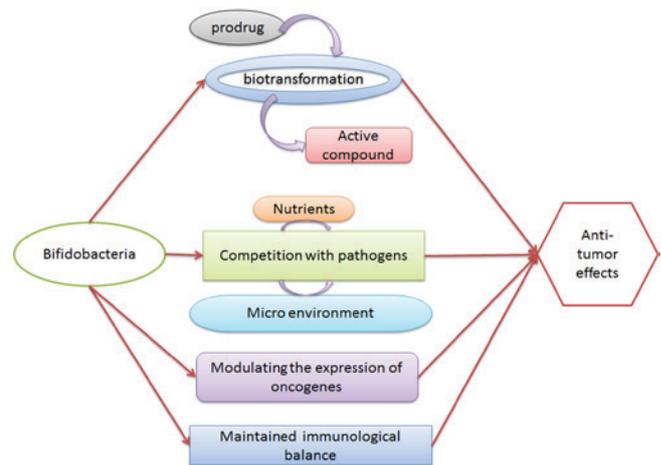


Figure 1. Schematic depiction of the various mechanisms involved in the antitumor effects of bifidobacteria.

azoxymethane were revealed to be decreased in animals fed with *B. longum* (27). *Bifidobacterium animalis* subsp. *lactis* may prevent colon carcinogenesis through inhibiting the formation of large ACF in mice colons (4). Another study revealed that the combined administration of *B. animalis* subsp. *lactis* and *Lactobacillus rhamnosus* with inulin in rats was able to decrease the occurrence of azoxymethane-induced malignant tumor types (28). A selenium-enriched *B. longum* strain may significantly inhibit tumor growth in tumor-bearing mice compared with negative control mice fed with 13% defatted milk (29). Three cell wall preparations from *B. longum* subsp. *infantis* revealed a high activity in tumor suppression and tumor regression tests on mice (30).

3. Competitive characteristics of bifidobacteria in a cancer microenvironment

Bifidobacterium has an apparent competitive advantage in the cancer microenvironment. The administration of *B. longum* resulted in a decrease in fecal pH, enabling the bacteria to outcompete commensal intestinal bacteria (31). The probiotic strain *B. breve* B632 possessed the ability to inhibit gas-producing coliforms, as observed when lowering of the amount of Enterobacteriaceae after 18 h of cultivation with *B. breve* B632-supplemented microbiota cultures, which accounted for 64% of bifidobacteria at the steady state, compared with in control cultures (32). Another study revealed that *B. breve* B632 and *B. breve* BR03 are able to inhibit certain gram-negative bacteria *in vitro* in order to integrate into the intestinal microbiota of children (33). Following feeding with a suspension containing 1x10⁸ live cells of *B. breve* B632 or *B. breve* BR03 daily for 21 days, a significant increase in total fecal bifidobacterial numbers and a parallel decrease in total coliforms was observed compared with the intestinal microbiota of children at the beginning of that study (33).

Secondly, the competitive exclusion of pathogenic microbiota by probiotics includes the competition for nutrients and adhesion at the intestinal mucosa. It has been reported that probiotics compete for limited nutrients available at the distal colon and grow at the expense of other bacteria (34-36). Non-pathogens may compete with pathogens by altering pH

and inducing metabolic changes (37,38). Vaginal lactobacilli may produce biosurfactants that are composed of a mixture of proteins, lipids and carbohydrates that help to displace dense mixed cultures of uropathogenic *Escherichia coli* and *Enterococcus faecalis* (39,40). The mechanism involved in anti-mutagenic activities is the ability of bifidobacteria to bind to the mutagens of microbial cells (41).

Thirdly, certain *Lactobacillus* strains may produce substances that kill or inhibit the growth of pathogens, including bacteriocins. Bacteriocins are molecules that may interfere with the cell wall structure and biosynthesis, forming pores in the target bacterial membrane resulting in permeabilization (42). In addition, lactobacilli may also produce hydrogen peroxide (H_2O_2), which has the potential to kill pathogens in the vagina via the production of free radicals (43), while they protect themselves from the toxic accumulation of H_2O_2 through the production of Fe^{3+} -activated extracellular peroxidase (44).

Finally, bifidobacteria have also been demonstrated to exhibit anti-mutagenic activities against heterocyclic amines, N-nitroso compounds and aflatoxins (45,46).

4. Gene and cytokine modulation: Cementing the function of the intestinal barrier

Bifidobacteria also exhibit antitumor effects via altering the expression of cancer-associated genes and cytokines. *B. longum* may suppress azoxymethane-induced colonic tumor types, and this effect was associated with a decrease in colonic mucosal proliferation and ornithine decarboxylase and ras-p21 activity (25,47,48). Animal studies have demonstrated that probiotic preparations consisting of bifidobacteria are able to decrease the activity of procarcinogenic enzymes, including *B. bifidum*, which may decrease the activity of β -glucosidase (49), and *B. longum*, which was revealed to lower β -glucosidase and β -glucuronidase activity (50). In addition, microbiota-associated inflammatory processes may contribute to carcinogenesis in the affected organs. For example, chronic *Helicobacter pylori* infection results in gastric cancer, and hepatitis B virus results in hepatic cancer (51,52).

However, the question of how they promote colon tumor formation remains unanswered. Patients with CRC exhibit bacteria adhering to tumor tissue (53) and have indirect evidence of bacterial invasion (54,55). Human gut commensal bifidobacteria strains have the ability to adhere to human epithelial cells (56). Exposure of *B. longum* subsp. *infantis* to human milk oligosaccharides resulted in an increased ability to adhere to intestinal cells and increase the expression of the anti-inflammatory cytokine interleukin (IL)-10 (57-59). In an animal model of post-infectious irritable bowel syndrome, which was established by infection with *Trichinella spiralis* for 8 weeks, *B. longum* intervention may increase the expression level of NLR family pyrin domain-containing 3 inflammasome and the downstream cytokines IL-8 and IL-1 β (60). For immune cells, *B. longum* BB536 serves an important function in the development and maturation of the immune system. Interferon γ (IFN- γ) secreting cells and the proportion of IFN- γ /IL-4 secreting T helper (Th) cells (Th1/Th2) were increased in newborn infants supplemented with *B. longum* BB536 (61). Wu *et al* (62) explored the immunological

mechanism of colonic carcinogenesis using enterotoxigenic *Bacteroides fragilis* (ETBF) and revealed a signal transducer and activator of transcription 3- and Th17-dependent pathway used in inflammation-induced cancer by ETBF. The beneficial effects of probiotics are thought to maintain the balance between types of T cell responses including T regulatory (Treg) and Th17 responses. The beneficial effects of probiotics are thought to keep balance between types of T cell response such as T regulatory (Treg) and Th17 responses (63,64). Th17 cells serve a key function in inducing tissue inflammation in autoimmune disease (65), and Treg cells function as master regulators of the immune response (66). However, *B. animalis* subsp. *lactis* was demonstrated to increase forkhead box P3⁺ mRNA, which defines the Treg lineage and expression in peribronchial lymph nodes, suggesting the induction of Treg cells by this strain (67). *Bifidobacterium*-treated mice revealed significantly limited tumor growth in comparison with non-*Bifidobacterium*-treated counterparts, which was accompanied by the induction of tumor-specific T cells in the periphery and an increased accumulation of antigen-specific cluster of differentiation 8 (CD8)⁺ T cells within the tumor (68).

Furthermore, the therapeutic effect of *Bifidobacterium* feeding was abrogated in CD8-depleted mice, indicating that the therapeutic effect of bifidobacteria on a tumor was dependent on antitumor immunity activity (68). In a study by Yin *et al* (69), *B. longum* was transfected by electroporation with pBV22210 containing IL-2 (*B. longum*-pBV22210-IL-2), and its inhibitory effect on transplanted tumor types in mice was examined. When cyclophosphamide was combined with *B. longum*-pBV22210-IL-2, the survival times of the mice were longer compared with any of them alone, indicating that *B. longum*-pBV22210-IL-2 has potent antitumor effects that may be enhanced when combined with chemotherapeutic drugs (69). Subjects using non-steroidal anti-inflammatory drugs compared with non-users had reduced numbers of *Collinsella* spp. and *Roseburia* spp., which are associated with the development of CRC (70). This means these bacteria require inflammatory niches to colonize the bowel wall (71). Therefore, an intact mucosa barrier is the key to protection from CRC development. First, *Bifidobacterium* may stabilize claudins at tight junctions (72). The administration of *B. longum* subsp. *infantis* BB-02 (3×10^6 CFU in 20 μ l) may decrease intestinal permeability in a necrotizing enterocolitis mice model by upregulating the expression of occludin and claudins 2, 4 and 7 (72). This function was also observed in enterocyte-like human colon adenocarcinoma cells and T84 cells (59,73). Secondly, bifidobacteria may affect mucosal metabolism and function, and be involved in colon cancer development via altering the expression of the intestinal differentiation factors, including Hes family basic helix-loop-helix (BHLH) transcription factor 1, atonal BHLH transcription factor 1 and Krüppel-like factor 4, which govern goblet cells (74).

5. Future treatment prospects using bifidobacteria

As bifidobacteria are non-pathogenic and anaerobic, and may selectively localize and proliferate in a hypoxic environment in a number of solid tumor types (75), transfected or constructed bifidobacteria as a novel delivery system for

specific genes is a promising therapeutic method for treating a tumor. For example, vascular angiogenesis is required for the growth and metastasis of a tumor (76). *B. longum* subsp. *infantis* transfected with *fms*-like tyrosine kinase receptor, a receptor for vascular endothelial growth factor, was verified to exert a potential effect on Lewis lung carcinoma in mice (77). Additionally, tumstatin (Tum) is an endogenous angiogenesis inhibitor (78). Wei *et al* (79) developed a delivery system for Tum using engineered *B. longum* (BL-Tum), used them to treat tumor-bearing mice, and revealed that the tumor in the BL-Tum group grew slowly with no metastasis observed. In another study, Nakamura *et al* (18) transfected *B. longum* with the plasmid Pbles100-S-eCD, which included the gene encoding cytosine deaminase. This transfected *B. longum*, which produced active cytosine deaminase that converted 5-fluorocytosine into 5-fluorouracil in hypoxic solid tumor types, resulted in 5-fluorouracil concentrations increasing proportionally with the number of the bacilli (with a baseline of 10³ CFU/ml). A similar study observed similar results in *B. longum* subsp. *infantis* (80).

6. Conclusion

In the present review, the role of bifidobacteria in the development of cancer was summarized, as transfected *Bifidobacterium* have been used to treat tumors in animal models (81). In the future, this novel technology may contribute to the discovery of treatment methods with fewer adverse effects for patients with cancer. However, further investigation of the use of bifidobacteria in the prevention and treatment of cancer is warranted.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

LC and HW collected the data and wrote the manuscript. GL, JY and FLi collected the data. YZ and FLu analyzed the data. YY conceived the idea for the study and revised the manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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