AKADÉMIAI KIADÓ

Acta Microbiologica et Immunologica Hungarica

69 (2022) 1, 1–12

DOI:
10.1556/030.2022.01650

REVIEW ARTICLE

The role of microbiota and immune system crosstalk in cancer development and therapy

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Received: November 2, 2021 • Accepted: January 6, 2022
Published online: January 25, 2022

ABSTRACT

Cancer is a multifactorial disease that is the second leading cause of death after cardiovascular disease in the world. In recent years, microbiota's role in the regulation and homeostasis of the immune system has been considered. Moreover, the immune system can affect the microbiota content. These interactions are critical to the functioning of the immune system. Numerous studies in animal and human models have shown the association of changes in microbiota components with the formation of an inhibitory microenvironment in the tumor and its escape from the immune system. Microbiota also plays a crucial role in the success of various anti-tumor treatments, and its modification leads to success in cancer treatment. The success of anti-tumor therapies that directly target the immune system, such as immune checkpoint blockade and T cell therapy, is also affected by the patient's microbiota composition. It seems that in addition to examining the patient's genetics, precision medicine should pay attention to the patient's microbiota in choosing the appropriate treatment method, and together with usual anti-tumor therapies, microbiota may be modified. This review discusses various aspects of the relationship between microbiota and anti-tumor immunity and its successful treatment.

KEYWORDS

microbiota, cancer, immune system, dysbiosis, cancer therapy, precision medicine

INTRODUCTION

Cancer is a term to describe a disease caused by uncontrolled growth and division of the cells. Less than 10% of all cancers are hereditary, while acquired somatic mutations and environmental factors cause the remainder. Multiple etiological factors may cause carcinogenesis, including chronic infections, dietary factors, obesity, inhaled pollutants, tobacco use, or autoimmunity [1]. The universal principle of all these factors is chronic inflammation caused by different inducers, such as classical inflammations due to infection and injury, which are associated with tissue malfunction [2]. The infection has been accepted as the cause of chronic inflammation and susceptibility to cancers, so that 16.1% of all new cancer cases in 2008 were associated with microbial infections [3]. Only ten from the estimated 3.7×10^{30} microbes in the world [4] are designated by the International Agency for Cancer Research (IACR) as a carcinogen for humans. Four main mechanisms have been suggested for the carcinogenicity of the microbes [5] as follows (Fig. 1):

1. **The integration into the host genome.** Ojesina and colleagues [6] have shown that *Human papillomaviruses* (HPV) selectively integrates within or close to several genes involved in cervical carcinomas. Also, at the site of HPV integration, the gene expression levels increase significantly, which could be associated with an increase in the gene copy number.

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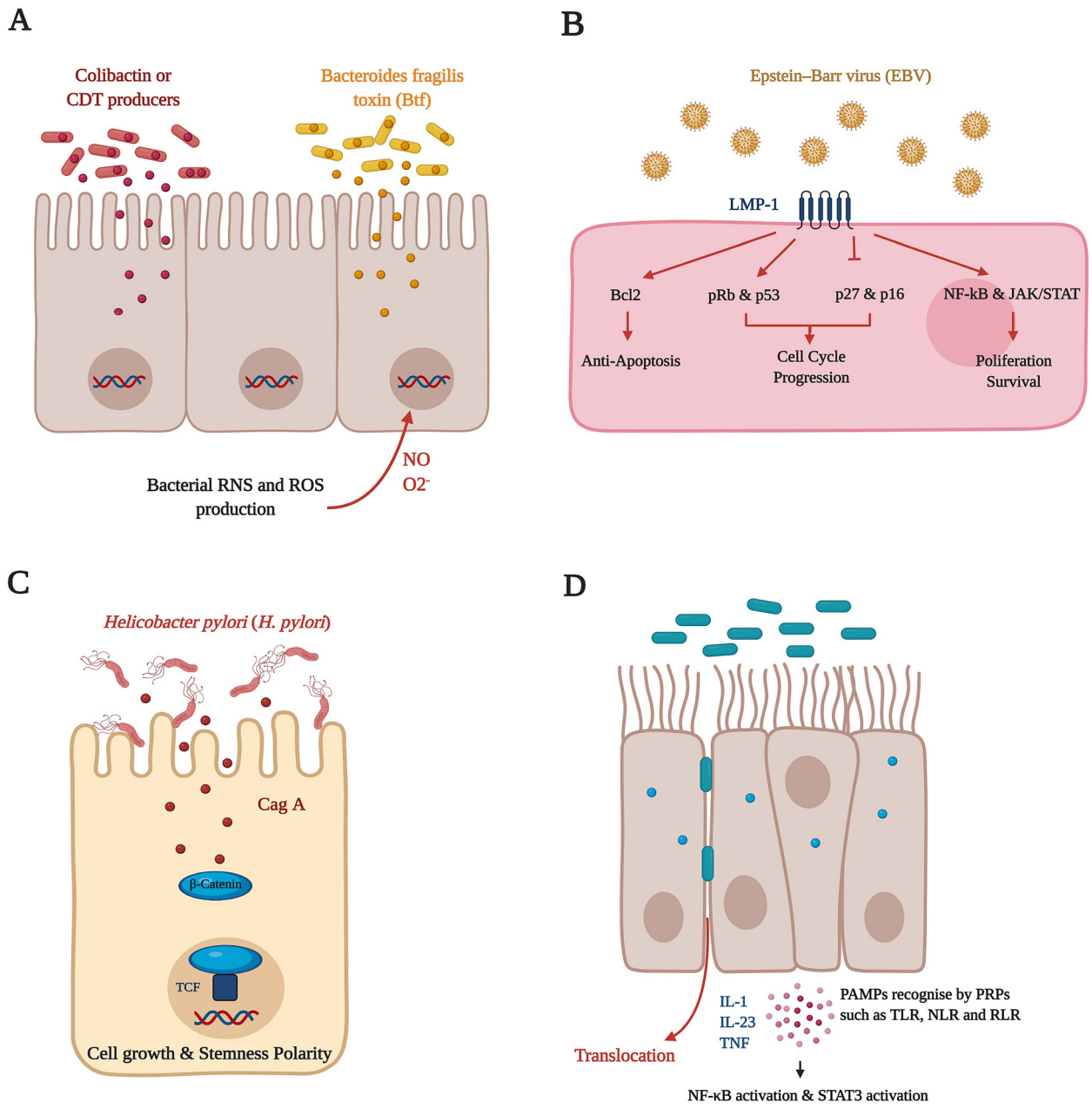

Fig. 1. Mechanisms by which microbiota impacts cancer development and progression. A) The bacterial toxin (such as colibactin, CDT) can directly damage DNA. *Bacteroides fragilis* toxin (Bft) can indirectly damage DNA by inducing O_2^- and NO production in the host. B) It seems that LMP1 is responsible for the oncologic effects of EBV. LMP1 provides signals for cell proliferation (by activating NF-κB and JAK/STAT), cell cycle propagation (by activating pRb and p53 and inhibiting of p27 and p16), and anti-apoptosis (by increasing Bcl-2 expression). C) CagA from *H. pylori* directly gets injected into the cytoplasm and activates β-catenin, which in turn mediates the upregulation of genes involved in carcinogenesis. D) Engagement of PRRs upon loss of boundaries between the host and microbe induces chronic pro-inflammatory responses through NF-κB and STAT3 signaling

2. **Induction of transformation by damaged DNA and genomic instability.** Colibactin produced by *Escherichia coli* and *Enterobacteriaceae* and cytolethal distending toxin (CDT) produced by several *Proteobacteria* are examples of toxins that can directly break the double-strand DNA [7]. *Bacteroides fragilis* toxin (Bft) of *B. fragilis* can induce high levels of reactive oxygen species (ROS) and damage the host DNA indirectly [8] (Fig. 1A).

3. **Affecting resistance to cell death and proliferative signaling.** Latent membrane protein-1 (LMP-1) is the oncogene of *Epstein–Barr virus* (EBV) which activates Bcl-2, NF-κB, and JAK/STAT signaling pathways; hence, it inhibits p53-mediated apoptosis and promotes survival and proliferation of B-lymphocytes [9] (Fig. 1B). CagA protein of *Helicobacter pylori* with activates β-catenin which upregulates the expression of genes that contribute

to cellular proliferation, survival, migration, and angiogenesis [10] (Fig. 1C).

4. **Inducing chronic inflammation and attenuating immune responses.** Chronic inflammation can promote neoplasia by using various mechanisms, including the increase of host cell proliferation that causes an increase in the probability of mutation, angiogenesis, escape from programmed cell death, and metastasis [11] (Fig. 1D). Many of the cancer-associated microbes, such as *Fusobacterium nucleatum* in colon cancer, activate the NF-κB signaling in the tumor microenvironment [12]. NF-κB and STAT3 were shown to be constitutively active in many cancers and were associated with the expression of genes involved in cancer-associated inflammation and cancer progression [13, 14]. The human immunodeficiency viruses (HIV) attenuate the immune responses by targeting CD4+ T cells, macrophages [15], and dendritic cells [16], which increase the risk of many cancers induced by oncogenic viruses [17].

The presence of such carcinogenic pathogens alone does not lead to the development of cancer. Numerous pieces of evidence have shown that microbiota plays an essential role in this regard. Microbiota is a collection of all commensal microorganisms that live in or on the host's body in a symbiotic relationship [18]. This symbiotic relationship depends on the anatomical separation of microbial entities from the host compartment by anatomical barriers. The destruction of these barriers can cause inflammation and diseases such as cancer. The microbiota plays an essential role in maintaining the barrier by maintaining the epithelial cell turnover and suppressing pathogens growth by competing for food sources [19]. Moreover, tumor growth requires the creation of a suitable microenvironment that suppress immune responses. Microbiota composition can affect the tumor microenvironment by altering tissue metabolism as well as differentiation and function immune system [20].

Gastrointestinal tract contains 99% of the microbiota mass, which exerts local and distant effects. Therefore, gut microbiota plays a crucial role in maintaining overall health and metabolic status. [19]. Change in microbiota compositions under pathogenic conditions (terms dysbiosis) reduces the protective and outgrown species by invasive inflammatory properties, known as pathobionts [21]. Dysbiosis can cause inflammation and imbalance in homeostasis, leading to cancerogenesis [17]. For instance, by eliminating *H. pylori*, the most known cause of gastric cancer, the probability of cancer development still exists. Several studies have confirmed the relationships of many cancers and the microbiota, including ovarian [22], head and neck squamous cell carcinomas [23], colorectal [24, 25], lung [26], and breast [27] cancers.

Microbiota can directly contribute to establishing metastatic tumors by traveling to distant areas with primary tumor cells. In colorectal cancer, the microbiome in metastases corresponds to the primary tumor, especially for *F. nucleatum*, one of the most prevalent bacteria in colorectal cancers. In mouse xenografts of human primary colorectal adenocarcinomas, a decrease of *Fusobacterium* load by antibiotic treatment reduced the tumor growth [28].

In recent years, the relationship between microbiota and cancer, known as the "oncobiome," has gained interest. This review discusses microbiota's role in the development and progression of cancer, the effects of microbiota on the immune responses and cancer treatment, and the future of microbiota in personalized medicine.

IMPACT OF MICROBIOTA ON ANTITUMOR IMMUNITY

The immune system prevents tumors by three primary functions. 1) By the elimination of viral infections, it can protect the host from the virus-induced tumors. 2) By removing the pathogens and suppressing inflammation, it prevents the formation of tumorgenesis-induced conditions by inflammation. 3) By specific recognition of tumor-specific antigens (TSAs), it can eliminate the tumor cells. The latter process is referred to as cancer immunosurveillance [29].

Furthermore, the immune system contributes to the tumor immunogenicity. The immune system has dual effects on developing tumors so that the cancer immunosurveillance hypothesis is refined into the cancer immunoediting. This dynamic process comprises three distinct phases: elimination, equilibrium, and escape (Fig. 2). In the elimination phase, that also known as cancer immunosurveillance, innate and adaptive immunity work together to recognize and destroy the nascent transformed cells so long before they become clinically apparent as a tumor mass [30].

Tumor cell variants always do not fully get eliminated by the immune system, but their growth gets controlled. Indeed, tumors enter into the equilibrium phase of the cancer immunoediting that tumor cells become functionally dormant and remain clinically unapparent for the life of the host. There is a dynamic interaction between the immune system and tumor cells in the equilibrium phase. Adaptive immunity contributes to this phase, and innate immunity does not play any role. Although there is a robust antitumor immunity, the tumor cells do not entirely get eliminated. Therefore, new variant cells are created that carry more mutations and low immunogenicity, that are more resistant to immune attack [31]. Three possibilities exist for a tumor that has entered the equilibrium phase: (1) eventual elimination by the immune system, (2) permanent maintenance by the cellular and molecular controls of immunity, or (3) escape from immune pressure and transit to the final escape phase of the immunoediting process [32].

Genetic and epigenetic changes in tumor cells make them resistant to immune detection and elimination. In the escape phase, these tumor cells can escape from antitumor responses and grow in these situations. Moreover, the immune system plays an active role in tumor progression by selecting low immunogenic and more aggressive tumor

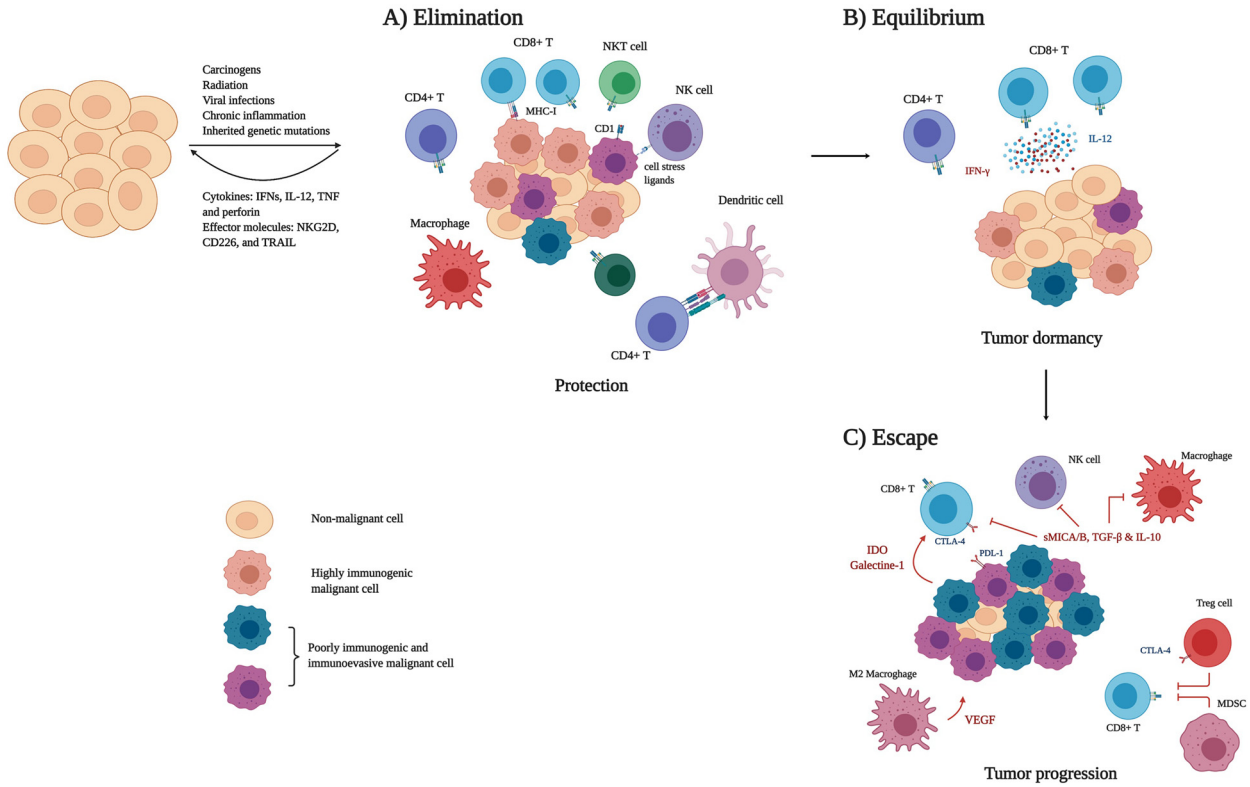

Fig. 2. Cancer immunoediting. Cancer immunoediting includes three phases; elimination, equilibrium, and escape, that function independently to control or shape cancer. A) During the elimination phase, innate and adaptive immunity recognize the transformed cells and destroy them before tumors become clinically detectable. B) If the transformed cells do not entirely get destroyed by antitumor immunity, these cells survive and enter the equilibrium phase, in which adaptive immunity prevents the tumor outgrowth and edits the cellular immunogenicity. C) Poor immunogenic tumors can enter the escape phase, where these variants escape from recognition and killing by immune cells and progress to clinically detectable. DC, dendritic cell; MDSC, myeloid-derived suppressor cell; MHCI, MHC class I; NK cell, natural killer cell; NKT cell, natural killer T cell; PD- L1, programmed cell death 1 ligand 1; TAM, tumor-associated macrophage; Treg cell, regulatory T cell

variants, suppressing antitumor response, and enhancing tumor cell proliferation.

Local or systemic alterations in the microbiome may impact the immunosurveillance and, consequently, lead to cancer's clinical outcome. According to the cancer hygiene hypothesis, the increase in particular cancer incidence is linked to the decreased exposure to specific microorganisms, and increased hygiene [33].

The influence of microbiota on anti-tumor immunity can be divided into two categories: antigenicity and adjuvanticity. Microbial proteins may be similar to the tumoral epitopes and elicit immune responses through antigenic mimicry or cross-reactivity. Aligning peptide sequences of TSAs with those from the microbiome showed significant homologies. For example, the $MAGE6_{172\text{-}187}$ epitope from melanoma is a highly homologous peptide within the *Mycoplasma* penetrans HF-2 permease (MPHF2) protein [34]. The adoptive transfer of memory Bf-specific Th1 cells into mice can reduce the growth of MCA205 fibrosarcomas [35].

Although T cells often primed with DC-presenting microbial antigens in intestinal sites (that are distinct from tumor sites) and educated for expression of tissue-specific homing receptors for local trafficking of T cells, the growing tumors could produce a high amount of chemokines and chemokine gradients that attract T cells primed in the gut toward tumor sites [36]. Furthermore, the antigen-specific effector and memory B and T cells primed locally in mucosal sites can seed other musical or lymphoid tissues that protect the host against the pathogens, regardless of the entry sites [37].

An alternative mechanism by which microbial antigens can trigger T cells activation via cross-reaction is their translocation or even the entire microorganisms from the intestine to other secondary lymphoid tissues. Effector T cells then migrate to the tumor sites and contribute to the immunosurveillance [36]. Peptidoglycans of the bacterial cell wall that shed during bacterial deviation can be recognized through widely expressed receptor beyond mucosal sites and regulates several host immune functions and physiology [38]. Furthermore, $CD103^+$ $CD11^+$ CDs in lamina properia capture the microbial antigens and migrate to the draining lymph nodes to activate the naive T cells [36].

Microbiota is not always linked to antigenic mimicry and provides non-antigenic co-stimulations that lead to the bystander activation of TSA-specific T cells and will guide them to differentiate effector CD4+ (such as TH1, TH2, TH17, or Treg) and CD8+ T cells [36]. By activating the

innate immune receptors (PRRs), microbes can induce cytokine production that regulates the tendency to inflammatory, immunostimulatory, or immunosuppressive reactions. The innate immune cells' requirement for antitumor immune surveillance has been evidenced for DCs, macrophages, and NK cells [36]. In germ-free mice, the function of innate immune cells was reduced, compared to the conventional mice, and IL-10 was secreted in response to LPS instead of TNF [39]. Microbial stimulation of DCs from germ-free mice expressed low levels of IFN-I, IL-6, TNF, IL-12, and IL-8 genes, and DCs and macrophages from these mice did not provide NK cell priming signals [40].

TLRs are essential PRRs that recognize PAMPs and DAMPs and can regulate the antitumor immune responses. Signaling of LPS receptor (TLR4) was essential for the outcome of total body irradiation (TBI) in mice. Radiation causes the translocation of microbiota from the gut into mesenteric lymph nodes and increases the sera's LPS levels. Immature DC is most likely LPS-responding cells and capable of active transferred tumor-specific $CD8^+$ T cells. Knockout of LPS signaling components (including CD14 or TLR4), antibiotic sterilization of the gut, or inhibition of LPS by polymyxin B reduced the destruction of tumors by the adoptively transferred cells [41].

The link between TLR5 signaling (flagellin receptor) and malignancy is highly dependent on the cancer type. $TLR5^{R392X}$ polymorphism that abrogates flagellin responses is associated with accelerated malignant progression in patients with luminal breast cancer, but in ovarian cancer patients, it increased the overall survival [42].

Several studies showed that Th17 cells and their cytokines are involved in intestinal tumorigenesis. Moreover, certain components of the gut microbiota may modulate Th17 cell responses [43]. Depending on the cancer type, IL-17A shows either pro- or anti-tumor activities. Th17 cells destroyed the advanced B16 melanoma even better than Th1 cells [44] but promoted the tumorigenesis in colorectal cancer [45]. Ablation of IL-17A in mice strains susceptible to the spontaneous intestinal tumor ($APC^{min/+}$) significantly reduced the tumor development [46]. IL-17A controls the ability of Treg to inhibit the intestinal tumorigenesis in these mice [47].

The gut microbiota may specifically induce $Foxp3^+$ Treg cells regulatory T cells (Tregs) and type 1 regulatory T (Tr1) cells that produce the anti-inflammatory cytokine IL-10. The Intestinal microbiota only impacts the colon Treg population, not the small intestine. IL-10 can control the proliferation of Th17 cells and the production of IL-17A that can have anti-tumorigenic activities in the intestine. For example, *Lactobacillus*, *Bifidobacterium*, and *Clostridium* may induce Treg and Tr1 cells [48].

MICROBIOTA AND ANTI-CANCER THERAPY

The composition of microbiota may impact the efficacy and toxicity of anti-cancer therapies including, immunotherapy, chemotherapy, immune checkpoint blockade, and T cell therapy.

Immunotherapy: TLR9 is the receptor for unmethylated CpG motifs present in microbial DNA, and its signaling induces the expression of pro-inflammatory cytokines. In humans, TLR9 engagement with synthetic CpG oligonucleotides (ODN) on plasmacytoid dendritic cells and B cells enhances the immune response. Although CpG-ODN, as an adjuvant, has been used for the human vaccine against malaria and Hepatitis B virus, it was not effective in cancer patients as a standalone therapy [49]. In mice, all myeloid cells express TLR9, intra-tumoral injection of CpG-ODN alone, or especially with anti-IL-10 receptor antibodies, which enhance the TSA-specific immune response and are effective against tumors [50]. The gut microbiota impacts the CpG-ODN immunotherapy. In antibiotic-disrupted microbiota or germ-free mice, tumor-infiltrating myeloid cells produce low levels of TNF and IL-12 after CpG-ODN/anti-IL-10R treatment. Commensal microbiota by activating myeloid cells induces these cytokines production in response to treatment. Furthermore, through TLR4 activation, microbial products may impact tumor-associated myeloid cells' priming for TLR9-dependent response to CpG-ODN [51]. The microbial composition showed a positive or negative correlation with the immune response after CpG-ODN/anti-IL-10R treatment. For example, TNF produced by the tumor cells correlated positively with Alistipes genera and negatively with Lactobacillus genera abundance in the feces [52].

Chemotherapy: Cyclophosphamide (CTX) is a successful anti-cancer alkylating drug commonly used in combination with other therapies to target tumor cells [52]. Due to their suppressive properties at high doses, CTX is used in bone marrow transplantation and autoimmune disorders [53]. A low dose of CTX promotes anti-tumor immunity through inhibiting the Treg cell function and activation of adaptive immunity that induces immunogenic cell death [54]. The therapeutic efficacy of CTX correlates with gram-positive commensal bacteria that translocated into secondary lymphoid organs (segmented filamentous bacteria, *Lactobacillus johnsonii*, *Lactobacillus murinus,* and *Enterococcus hirae*) and provide a favorable immunological environment for the development of Th17 cells by anti-tumor activity. Furthermore, gram-positive bacteria deletion with vancomycin A reduced CTX therapy's efficacy in mice inoculated with MCA205 tumors by preventing the CTX-induced accumulation of Th17 in the spleen [55].

The microbiota also affects the platinum salts (including oxaliplatin and cisplatin) in cancer treatment. Platinum salts interact with DNA, causing DNA damage through intra-strand cross-link adducts. This interaction activates several signaling pathways and leads to the activation of apoptosis. Also, oxaliplatin induces an anti-tumor T cell immunity and causes immunogenic cell death. Similar to CpG-ODN, germ-free mice failed to respond to oxaliplatin treatment. However, its anti-tumor effect is independent of TNF. ROSs are essential to induce DNA damage and apoptosis in response to platinum salts, and the gut microbiota primes myeloid cells to release ROS [51]. Microbiota can also contribute to chemotherapy drug metabolism, which impacts the effectiveness and side effects of drugs. Human

microbiota can directly modulate the gene expression of enzymes involved in drug metabolisms, such as cytochrome P450s (CYPs), dehydrogenases, and carboxylesterases [56]. For example, the gut microbial β-glucuronidases hydrolyze the inactive metabolite of irinotecan used in the metastatic CRC and cause side effects (*e. g.* severe diarrhea) in patients. Inter-patients variation in the efficacy of the anti-CRC drugs, such as 5-fluorouracil (5-FU) and 5-fluoro-2′-deoxyuridine (FUDR), and the topoisomerase I (topo-I) inhibitor camptothecin (CPT) was not only affected by the host genetic but also by the gut microbiota [57, 58]. Recently it has been reported that the microbiota dysbiosis reduced the anti-tumor efficacy of 5-FU treatment. 5-FU is a cytotoxic agent that is the most common and standardized chemotherapeutic agents in colorectal cancer. The microbiota's initial gut composition is a crucial factor driving the host response to the anti-tumor drug of 5-FU [59].

Immune checkpoint blockade: One of the essential tumor escape mechanisms is the upregulation of immune checkpoint molecules, such as CTLA4 and PD1, that act as negative regulators for T cell activation. Targeting these immunomodulatory molecules on T cells (or their ligands) with the immune checkpoint inhibitor treatments enhances the anti-tumor responses. Despite successful treatment with these agents, a significant number of patients did not show any response or may have created a nondurable reaction [60].

The treatment of patients with melanoma by ipilimumab (anti-CTLA4) has a positive correlation with the colonization of *Faecalibacterium* and other *Firmicutes* that are associated with the low frequency of $\alpha4^+$ $\beta7^+$ T cells and Treg cell in circulation, as well as low levels of systemic inflammatory soluble proteins, such as IL-6, IL-8, and sCD25. In contrast, the presence of *Bacteroidetes* (mostly *Bacteroides* genus) in the gut microbiota was associated with the inadequate responses to ipilimumab in these patients [61]. Frankel and colleagues showed that the efficacy of immune checkpoint therapy with anti-PD-1 and anti-CTLA4 was associated with the colonization of the gut microbiota by *Faecalibacterium prausnitzii*, *Bacteroides thetaiotamicron*, and *Holdemania filiformis*. Moreover, enrichment for *Dorea formicogenerans* correlated with increasing the anti-PD-1 response (Fig. 3) [62].

In melanoma, patients with high diversity and abundance of *Ruminococcaceae* and *Faecalibacterium* in the gut microbiota responded better to anti-PD-1 therapy. It was associated with an increase of $CD8^+$ cells in the tumor site and $CD4^+$ and $CD8^+$ T cells in the systemic circulation that showed cytokine response to anti-PD-1 therapy. In contrast, patients with low diversity and high abundance of *Bacteroidales* in the gut microbiota showed an inadequate response to anti-PD-1 therapy and impaired systemic and anti-tumor immunity due to the high frequency of Tregs and MDSCs in systemic circulation and blunted cytokine responses [63]. Matson and colleagues showed a significant association between particular bacterial species in the gut microbiota (included *Bifidobacterium longum*, *Collinsella aerofaciens*, and *Enterococcus faecium*) and clinical response in responder patients with melanoma to anti-PD-1 therapy. Transfer of fecal material from these responder patients to germ-free mice restored the response to anti-PD-L1 therapy. It improved tumor control by decreasing of Treg cells and the increase of DC and augmented the Th1 responses [64]. Concomitant antibiotic therapy changed the gut microbiota compositions and inhibited the response to anti-PD-1/PD-L1 therapy in patients with advanced non–small cell lung cancer (NSCLC) and renal cell carcinoma (RCC). Metagenomics analysis of stool samples from patients with NSCLC and RCC showed a correlation between anti-PD-1/PD-L1 therapy and the relative abundance of *Akkermansia muciniphila*. Moreover, clinical outcomes during PD-1 therapy correlated with Th1 and IFN-γ^+ $CD8^+$ T cell reactivity from T cells of NSCLC patients against *A. muciniphila*. Fecal microbiota transplantation from responder patients overcame resistance to anti-PD-1 therapy in antibiotic-treated or germ-free mice, dependent on IL-12 and an increase of $CCR9^+CXCR3^+CD4^+$ T cells into tumor microenvironments [65].

T cell therapy: Adoptive T cell therapy (ACT) (*i. e.*, adoptive transfer of in vitro expanded tumor-infiltrating lymphocytes (TIL) or chimeric antigen receptor (CAR) T cells) is another branch of immunotherapy that showed great success in clinical trials. However, there is still an occurrence of treatment failure that may be due to the peripheral tolerance and immune evasion by the tumor. As aforementioned, the microbiota may affect the T cell phenotype and function. The efficacy of ACT in tumor-bearing mice changes based on the abundance or presence/absence of *Bacteroidetes* and *Firmicutes* in the gut microbiota. Although the treatment with an antibiotic before ACT reduces the tumor progression, it is dependent on the type of antibiotic (s). Overall, the efficacy of ACT is associated with an increase in systemic CD8α+ DCs and IL-12p70 levels [66]. In the patients treated by CAR-T cells, the presence of *Oscillospiraceae, Ruminococcacaeae*, and Lachnospiraceae in the gut microbiota was associated with a complete response; however, *Peptostreptococcaceae* was more abundant in patients with no complete response [67].

THE ROLE OF MICROBIOTA IN THE FUTURE OF PRECISION MEDICINE OF CANCER

At the beginning of present century, with our understanding of the human genome, precision medicine was introduced to treat patients based on their genomic properties. Microbiome states are complex and highly individual-dependent, which can be rapidly changed to respond to environmental circumstances and stresses. Many bacteria and microbiota are important in both health and disease. Therefore, understanding the microbiota composition may be crucial in the individualized treatments [68]. As we discussed earlier, there is a relation between microbiota with cancer development, immune responses, and the outcome of cancer treatment. Therefore, the microbiome (especially gut) can be used as a biomarker for early detection or determination of

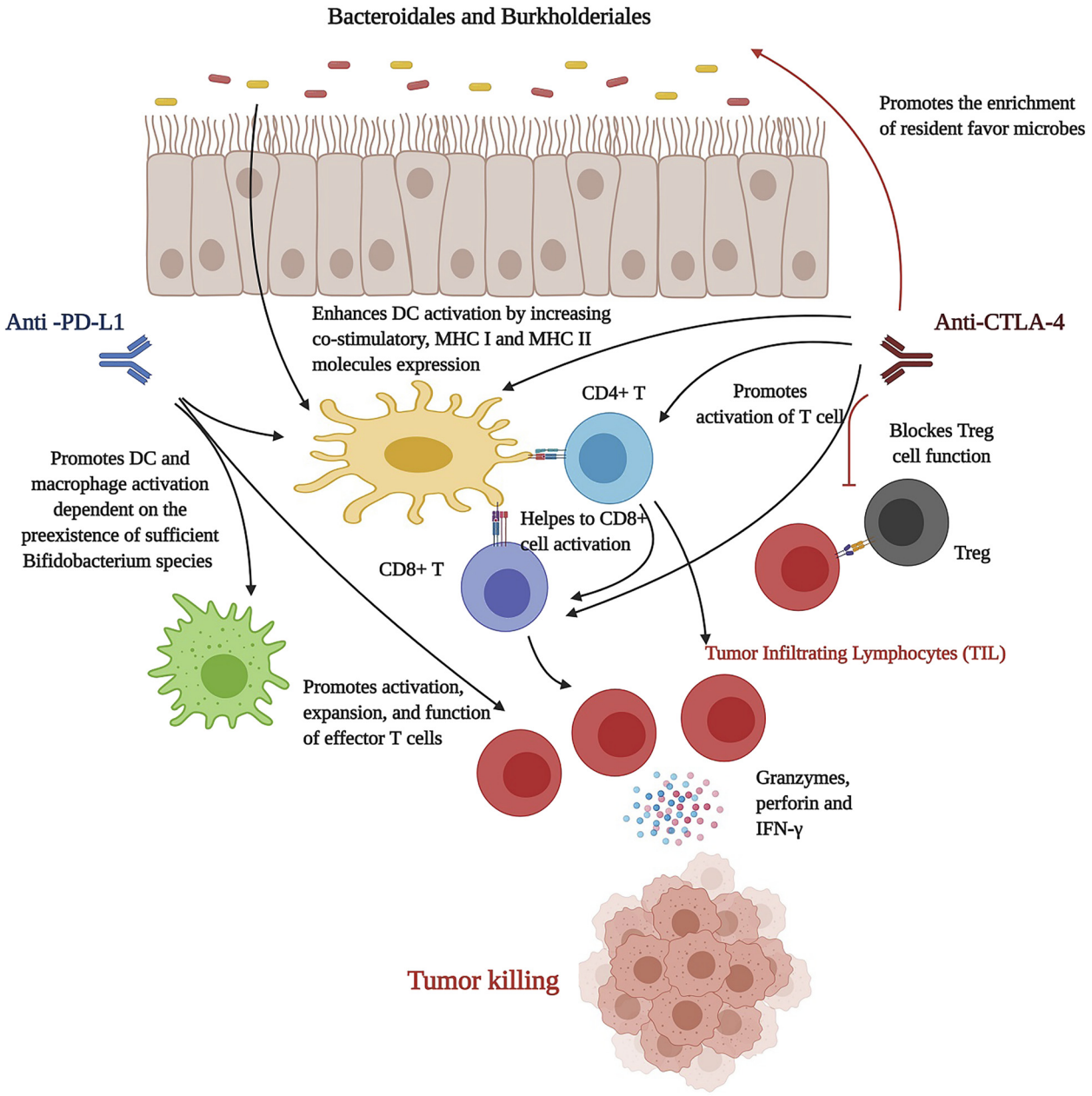

Fig. 3. Impacts of the gut microbiota on anti-cancer immunity. The efficacy of both anti-CTLA-4 and anti-PD-L1 therapies depends on the gut microbiota. *Bacteroidales* and *Burkholderiales* species enhance the efficiency of anti-CTLA-4 therapy; moreover, this therapy increases these useful bacteria. Anti-CTLA-4 promotes the activation and function of effector T cells and DCs and blocks Treg cell inhibitory function. Anti-PD-L1 therapy depends on the pre-existing *Bifidobacterium* species and enhances DCs and macrophages activation. Anti-PD-L1 promotes the activation, expansion, and function of the effector T cell

cancer and targets therapeutic intervention for improving cancer therapy in precision medicine [69]. Correction of microbiota in patients may be a useful way for the improvement of cancer treatment.

Specific antibiotics: Antibiotic treatment has off-target effects and massively changes the microbiota composition in humans. Pathogens targeting specific antibiotics or phages create new opportunities for precision medicine to modulate the microbiota. Species-specific enzyme inhibitor and antimicrobial molecules are the targets to design specific antibiotics. For example, a synthetic microbicidal peptide (STAMPs) is designed to target *Streptococcus mutans* precisely [70]. Moreover, the use of a specific bacteriophage cocktail reduced *E. coli* equally to ciprofloxacin treatment (a broad-spectrum antibiotic) without having a significant impact on microbiota [71].

Probiotic: another way to manipulate the microbiota is an increase in the level of beneficial bacteria instead of the elimination of harmful bacteria. Probiotic supplementation and fecal microbiota transplantation (FMT) are used for this purpose. The FAO/WHO defines a probiotic as "live microorganisms which, when administered in adequate

amounts confer a health benefit on the host" [72]. Probiotics can exert preventive and therapeutic roles in several ways in cancer, including (Fig. 4):

1. Negation of dysbiosis by modulating the gut microbiota; for instance, several gram-positive probiotics (*e. g.*: *Bifidobacterium* spp. and *Lactobacillus* spp.) can inhibit the growth of several gram-negative pathogens (*e. g.*: *H. pylori* and *Salmonella enterica* [73]) by the synthesis of antimicrobial peptides, acetic, lactic and propionic acid [74].
2. Reducing the activities of pro-carcinogenic enzymes (*e. g.*: *β* –glucuronidase, azoreductase, and nitroreductase) that may produce pro-carcinogenic substances in the gut [75]. *Lactobacillus acidophilus* oral supplement reduced the activities of these enzymes in patients with cancer [76].
3. Inactivation of carcinogens. Certain *Lactobacillus* spp. can bind to and reduce the genotoxic and mutagenic activities of potent carcinogens, such as Trp-P-2 (3-amino- 1-methyl-5H-pyrido (4, 3-*β*) indole) [77] and MNNG (N-methyl-N′-nitro-N-nitrosoguanidine) [78].
4. Prevention of DNA damage. Probiotics can decrease the effects of DNA damaging mutagens, such as 2-dimethylhydrazine (DMH) [79], N-nitrosodimethylamine (NDMA) [80], and 2-amino-alpha-carboline (AAC) [81]. Moreover, probiotic *Lactobacillus* spp. can bind to heterocyclic amines (HCA) generated in cocked meat and prevent the formation of DNA adducts. For example, some *Lactobacillus* spp. could bind to PhIP (2-Amino-1-methyl6 phenylimidazo [4,5b] pyridine) that has been classified by the IARC as potentially carcinogenic to human (Group 2B) [82].
5. Modulation of the immune and cell responses. *Lactobacillus rhamnosus GG* can reduce the risk of colon cancer by decreasing the expression of inflammatory genes (*e. g.:* NF-*κ*B-p65, COX-2, and TNF, *β*-catenin and Bcl-2), and increasing of pro-apoptotic genes (*e. g.:* Bax and p53) [83]. Furthermore, a mix of *L. acidophilus*, *Bifidobacteria bifidum*, and *Bifidobacteria infantis* (LBB) increased the TLR2 signaling that was associated with a decrease in the expression levels of *β*-catenin and the relative frequency of pathogenic bacteria, such as *Escherichia, Pseudomonas, Helicobacter, Chlamydia* in the gut. LBB also enhances the intestinal barrier by inducing MUC2, ZO-1, and occludin expression and inhibits the inflammation by decreasing the TLR4 and COX-2 [84]. *Bacillus polyfermenticus* probiotic not only inhibit the growth of human colon cancer cells, including HT-29, DLD-1, and Caco-2 cells [85], but also increase the IgG production, which therefore modulates the number of total T, $CD4^+$ T, $CD8^+$ T, and NK cell in human [86]. Probiotics can enhance mucosal and systemic immunity. Administration of a mixture of *Bifidobacterium* spp., *Lactobacillus* spp. and *Streptococcus salivarius* (VSL#3) to rhesus macaques increased the frequency of $IL\text{-}23^+$ APC in colon and LNs that was

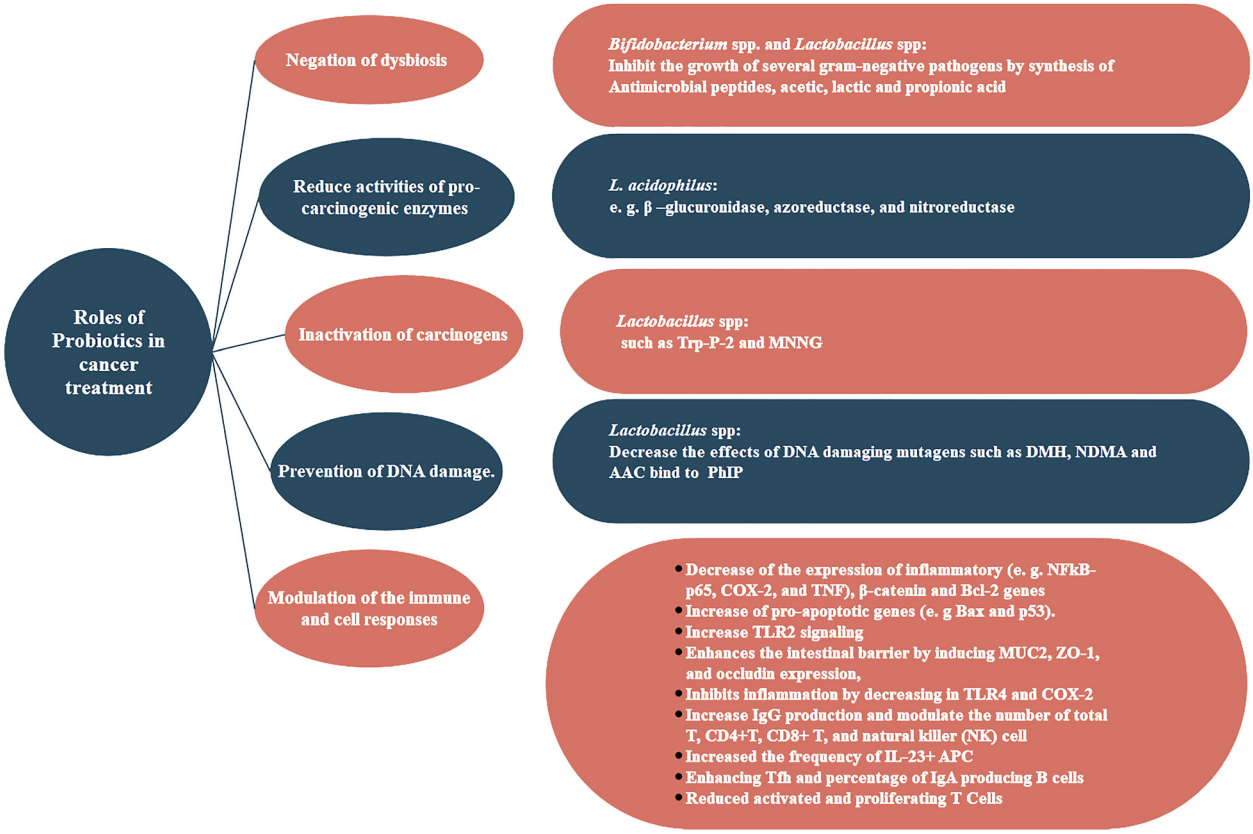

Fig. 4. Mechanisms of probiotic action in prevention and therapy of cancer

related to the enhanced percentage of Tfh and IgA producing B cells in both sites. VSL#3 also increased ILC3 frequency in the jejunum and colon. However, VSL#3 reduced the activity and proliferation of T cells in mucus [87].

Fecal microbiota transplantation: another way to target the intestinal microbiota is FMT that is the transplantation of gut microbiota from a healthy donor to a patient to restore intestinal microbial diversity [88]. In 2013, the FDA approved FMT to treat *Clostridium difficile* infection, which was not responsive to standard therapies [89]. In the colon cancer model, the transplantation of wild mouse microbiota was associated with the smaller tumor, reduced inflammation, and fewer metastases [90]. Several FMT in patients with hepatic encephalopathy reversed the intestinal dysbiosis and resulted in their improvement [91]. FMT could also restore the microbiota after irradiation and increase the survival rate of animals [92]. Autologous FMT restored microbiota diversity in humans and mice that were exposed to antibiotics and chemotherapy [93, 94].

CONCLUSION

As we discussed earlier, there is a strong relationship between microbiota and anti-cancer immunosurveillance and the outcome of cancer treatment. The presence of carcinogenic pathogens is not enough to create a carcinogenic environment. Dysbiosis helps cancer development through several mechanisms. Moreover, there is a crosstalk between microbiota and the immune system. The microbiota can enhance innate and adaptive anti-cancer immune response through mimics of tumor antigens and provide non-antigenic co-stimulations that lead to TSA-specific T cells' bystander activation. Therefore, dysbiosis can shift and reduce immune responses to cancer. The efficacy of anticancer therapies is also dependent on microbiota compositions. Therefore, microbiota correction may be a useful approach to enhance the treatment outcomes and reduce their cytotoxic and side effects.

Microbiome states are highly individual and rapidly changed to respond to environmental circumstances and stresses. Therefore, the microbiota composition determination, along with the genetic of the patients, makes an opportunity for precision medicine to select the proper treatment for each patient.

Financial support: No relevant financial support was provided.

Potential conflicts of interest: The authors have no conflicts of interest to disclose.

Patient content statement: This study does not include factors necessitating patient consent.

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