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The Emerging Role of gut Microbiota in Immunotherapy

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Abstract:

The gastrointestinal tract contains a diverse and dynamic population of microorganisms called the gut microbiota, which is essential for immune system regulation. Recent studies have highlighted that gut microbiota has a major influence on the effectiveness of cancer immunotherapy, particularly immune checkpoint antagonists. This review explores the mechanisms of specific gut microbiota, such as Bifidobacterium and Lactobacillus, to enhance innate and adaptive immune responses, including activation of dendritic cells, polarization of macrophages, and stimulation of CD8+ T cells. In addition, this review discusses therapeutic strategies aimed at manipulating the gut microbiota to enhance the effects of immunotherapy, including fecal microbiota transplantation (FMT), probiotics, and engineered microbiota. Although these approaches have promising potential, the challenges still remain with the variability of patients and microbiota manipulation. This review emphasizes how crucial it is to combine immunotherapy and the gut microbial community to create more individualized, effective cancer treatments, which will eventually improve patient outcomes and advance the area of cancer treatment.

Keywords: Gut microbiota; immunotherapy; microenvironment.

1. Introduction

It is becoming more widely acknowledged that the gut microbiome, a large and complex population of bacteria that live in the human gastrointestinal system, which plays a significant role in determining both health and disease. The gut microbiome is composed of trillions of microbial cells, including bacteria, viruses, fungi, archaea, and protozoa. The metabolism of nutrients and digestion are significantly influenced by the gut flora. In addition to these functions, the gut microbiota also contributes significantly to the development of the immune system in the host, especially in regulating innate and adaptive immunity. Studies have shown that the disruption of this delicate microbial balance (called dysbiosis) is a precursor to a range of malignant diseases, including autoimmune diseases, neurological diseases, and especially cancer. As a result, research is turning its attention to the complex link between the immune system and the gut microbiome, which has significant implications for the understanding and ability to treat a variety of diseases, including cancer.

One of the most important developments in contemporary medicine is cancer immunotherapy, which offers a completely new way to treat cancer. Unlike traditional therapies that directly target tumor cells, immunotherapy stimulates or restores the host's immune system to identify and destroy cancer cells. Immune checkpoint medications, which suppress the CTLA-4 and PD-1/PD-L1 pathways, have demonstrated notable effectiveness in treating a variety of malignancies, such as renal cell carcinoma, non-small cell lung cancer, and melanoma [1,2]. Although the success of immunotherapy, its efficacy varies widely among patients. Some patients experience significant benefits and others experience little improvement. This variability has prompted intensive research to identify biomarkers that can predict immunotherapy response and understand the underlying mechanisms that influence treatment outcomes.

More research has demonstrated recently that the anti-tumor benefits of immune checkpoint blockade can be increased when particular gut bacteria are present, and the therapeutic effect can be decreased when these microorganisms are absent [3,4]. These groundbreaking findings have prompted further research into the correlation between intestinal microbiota composition and immunotherapy response. For example, patients seem to respond better to PD-1 blocking medication when specific bacterial genera, such as Bifidobacterium pseudolongum and Lactobacillus johnsonii, are more abundant in their bodies [1]. On the other hand, dysbiosis of the gut microbiota is linked to poor clinical results. This review introduces the potential of integrating intestinal microbiota regulation into immunotherapy as a major advance in cancer treatment. Outlines therapeutic approaches combining intes-

tinal microbiota with immunotherapy by comprehending and making use of the intricate relationships between the immune system and the gut bacteria. As this field of study develops, intestinal microbiota-based therapies are likely to become an integral part of cancer treatment, which can change the way we approach immunotherapy and bringing new hope to cancer treatment.

2. Gut Microbiota Remodel the Tumor Microenvironment

2.1 Gut Microbiota Modulates Innate Immunity

2.1.1 Dendritic cells (DC)

DC are key antigen-presenting cells that initiate and regulate immune responses. Activated DCs move to lymph nodes, where they expose T cells to antigens and start the immune system's attack on malignancies. It has been demonstrated that the gut microbiota, particularly species like Bifidobacterium and Lactobacillus, promotes the maturation and activation of DC.

Oral Lactobacillus can increase the maturation and activation of dendritic cells (DC), thereby inducing the expression of cytokines, and are used as an immune-enhancing biological feed additive to treat suckling piglets with intestinal flora imbalance caused by infection. It has been reported that when lactating piglets were orally fed with Lactobacillus, the number of DC maturation markers increased significantly, which indicated that Lactobacillus has the ability to regulate DC maturation [5]. Oral supplementation of Lactobacillus also significantly increased the levels of IFN-γ, IL-10, and IL-4 proteins in the intestine of suckling piglets [5].

Oral administration of Bifidobacterium can increase DC activation, thereby promoting the secretion of interleukin 12 (IL-12) and enhancing the therapeutic efficacy of CTLA-4 inhibition and anti-PD-L1 therapy in mice with "unfavorable" intestinal flora. Studies have shown that oral Bifidobacterium can upregulate tumor cell death and suppress tumor growth by recruiting DC [6]. DC is an important source of secretion of anti-tumor factor IL-12, a protein that is important for regulating immune responses and actively. Through its role in the development of naive T cells into Th1 cells, IL-12 promotes the production of IFN-γ, which in turn increases the capacity of T cells and natural killer (NK) cells to eliminate cancerous or infected cells. According to research by Mager et al., Bifidobacterium pseudolongum and Lactobacillus johnsonii added to mice (with ICIs) bearing four distinct malignancies markedly increased the effectiveness of anti-CTLA4 and anti-PD-L1 immunotherapy [1]. Further evidence of the importance of Bifidobacteria and Lactobacilli in regulating innate immunity.

2.1.2 Macrophages

Key participants in the innate immune response, tumor-associated macrophages (TAMs) often exist in two primary states: pro-inflammatory M1 phenotypes and anti-inflammatory M2 phenotypes. M1 macrophages, which are activated by TNF-α and IL-12, are essential for both host defense and anti-tumor immunity. IL-4 and IL-13 stimulate M2 macrophages, which are involved in reducing inflammation and tumorigenesis. Both M1 and M2 macrophages have high plasticity. Thus, the balance and mutual conversion of M1 and M2 macrophages have become targets for tumor therapy. In the intestinal microbiota, Akkermansia muciniphila and Bacteroides fragilis have been shown that have the ability to increase the M1 polarization of macrophages.

A. muciniphila has the ability to induce macrophages to polarize to the M1 phenotype, which triggers the production of proinflammatory cytokines and corrects the M1/M2 macrophage balance in the tumor microenvironment of mice that are harboring tumors. Studies have found that after the use of A. muciniphila on RAW macrophages, a large number of surface markers of M1 phenotype macrophages, such as the expression of CD40 and CD8 were detected, indicatings that A. muciniphila stimulated the polarization of macrophage M1 phenotype [7]. Another study found that M1 and γδT cells in NASH mice were continuously enriched and their liver TLR2 expression was downregulated after being treated with A. muciniphila [8]. This demonstrates even further that A. muciniphila can encourage macrophage polarization toward the M1 phenotype.

Deng's group used ZY-312, an isolate of Bacteroides fragilis on bone marrow-derived macrophages (BMDM), and observed increased cellular expression of CD80 and CD86 and increased expression of cytokines such as IL-12 in macrophages [9]. This study demonstrated that Bacteroides fragilis had the ability to enhance the phagocytosis and polarization of M1 phenotype macrophages. In summary, the specific intestinal microbiota is crucial for regulating macrophage polarization and inhibiting or promoting tumor growth, which depends on the balance of M1/M2 macrophages within the TME.

2.2 Gut Microbiota Modulates Adaptive Immunity

CD8+T cells, known as cytotoxic T lymphocytes (CTLs), are a subset of T cells that are essential to the immune system's ability to fight cancer and infection. These cells are characterized by the presence of the CD8 glycoprotein

on their surface, which acts as a coreceptor to recognize antigens presented by infected or abnormal cells. Histocompatibility complex (MHC) class I molecules, which are expressed on almost all nucleated cells in the body, are recognized by CD8+T cells as a means of determining whether a cell is aberrant. A distinct T cell receptor (TCR) that is specific for a particular antigenic peptide presented by MHC class I is also expressed by each CD8+ T cell. Because of their specificity, CD8+ T lymphocytes are able to identify and eliminate cancerous or contaminated cells. Naive CD8+ T lymphocytes can be seen in lymphoid tissues and blood. They are activated by specific antigens presented by DC, which stimulatory molecules like IL-2, IL-12, and IFN-γ to help to activate CD8+ T cells. Activated CD8+ T cells typically use the fas-fasL route and secretion of cytokines to eliminate tumor cells or infected cells.

Oral administration of Bifidobacterium can promote tumor-specific CD8+T cell responses by increasing dendritic cell (DC) activation and enhancing the therapeutic impact of anti-PD-L1 therapy in mice with tumors. Studies have reported that oral administration of Bifidobacterium to TAC mice and JAX mice with melanoma resulted in a considerable rise in DC cells and T cells and stimulated the activation of CD8+T cells and the secretion of IFN-γ factors [3]. The combination of oral Bifidobacterium and anti-PD-L1 therapy significantly inhibited the growth of tumors in mice. Moreover, research by Lin et al. showed that IL-12 can promote the secretion of IFN-γ by activating CD8+ T cells while counteracting the negative regulatory effect of IFN-γ, which can restore the therapeutic ability of adoptive cell therapy (ACT) in tumor-bearing mice [4].

3. Gut Microbial Metabolite-Mediated Antitumor Immune Response

3.1 Short-Chain Fatty Acids (Scfas)

The fermentation of indigestible carbohydrates by gut microbes results in the production of SCFAs, such as acetate, propionate, and butyrate. SCFAs are absorbed by colonocytes and used as an energy source to support gut and organ health. These metabolites have attracted much attention for their ability to alter both innate and adaptive immune responses, especially in the tumor microenvironment.

One of the key mechanisms of SCFAs is the regulation of DC to anti-tumor. Studies have shown that SCFAs can improve DCs' capacity to deliver antigens, which promotes the growth and activation of CD8+ T lymphocytes specific to tumors [10]. In particular, butyrate has been found to increase MHC class I molecule expression on tumor cell surfaces and enhance their recognition by cytotoxic T lymphocytes (CTLs) [11]. When butyrate-producing Lactobacillus supernatants were added to human HT-29 cells, the cell cycle was stopped at the G2/M phase and cyclins B1 and D1 were significantly downregulated [12], which indicated that butyrate-producing Lactobacillus strains effectively inhibited tumor growth. Similarly, in the report of Chang et al. demonstrated that feeding butyrate-producing Butyricicoccus pullicaecorum to mice with CRC, which significantly reduced serum carcinoembryonic antigen levels while causing weight gain in mice [13]. In addition, butyrate can also treat CRC caused by p53 mutation by downregulating the expression level of chromosome segregation 1-like (CSE1L) [14].

SCFAs not only can enhance antigen presentation but also can modulate the inflammatory environment within tumors. Because of its anti-inflammatory qualities, butyrate can decrease the production of cytokines that promote tumor growth, including TNF-α and IL-6, while increasing the synthesis of cytokines that reduce inflammation, like IL-10 [15]. Furthermore, regulatory T cells (Tregs), which are typically overexpressed in the tumor microenvironment, are regulated by SCFAs. SCFAs can restore anti-tumor immune responses by inhibiting Tregs. Studies have shown that butyrate can restrict Tregs through its histone deacetylase (HDAC) inhibitory activity, which enhances the efficacy of immune checkpoint inhibitors [16,17]. This suggests that dietary interventions that increase SCFA production in the intestine have positive effect on immunotherapy for patients.

3.2 Bile Acid

Bile acids (BA) are the main metabolites of intestinal flora. They are essential to the breakdown and absorption of fat. In addition to their digestive function, BA has been determined as important signaling molecules that regulate metabolic processes and immune responses. BA can be divided into two types: primary BA and secondary BA. Cholesterol is the primary source of liver synthesis for primary BA, which includes cholic acid (CA) and chenodeoxycholic acid (CDCA) in humans. Primary bile acids like deoxycholic acid (DCA) and lithocholic acid (LCA) work on the gut microbiota to create secondary bile acids in the intestine.

Bile acids regulate macrophage polarization and the switch between macrophage M1 and M2 phenotypes by activating the farnesoid X receptor (FXR). M2 macrophages control tissue remodeling and regeneration, whereas M1 macrophages have the ability to destroy tissue and interfere with wound healing processes. Studies have shown that FXR agonists increase the expression of retinoic acid receptors while promoting M2 macrophage

polarization, which enhances the effect of macrophage M2 phenotype polarization in chitin-treated mice [18]. Another study revealed that the bile acid derivative 6β-Ethyl-3a,7b-dihydroxy-5b-cholan-24-ol (BAR501) can activate the secondary bile acid receptor GPBAR1 [19]. Activated GPBAR1 stimulates the transformation of M1 macrophages into M2 macrophages, which eliminates the inflammation accumulated in the M1 phenotype and restores the immune dysfunction of colitis mice [19]. Therefore, bile acid treatment can be considered as a therapeutic approach to support the remodeling of tissues and repair mediated by M2 macrophages.

Bile acids also have context-dependent impacts on immunological responses. Some bile acids may promote tumor growth, such as deoxycholic acid (DCA), which tends to increase cancer risk due to its proinflammatory and genotoxic effects [20]. Therefore, understanding the specific roles of different bile acid species in cancer is critical for developing targeted therapies that exploit bile acid signaling to improve the effectiveness of immunotherapy.

In summary, SCFAs and bile acids represent two important classes of gut microbial metabolites with significant impacts on anti-tumor immunity. SCFAs create an immune environment that favors effective anti-tumor responses by regulating DC function, cytokine production, and Treg activity. On the other hand, bile acids influence immune cell function and gut microbiota composition, which further shapes the tumor microenvironment. The goal is to create new treatment approaches and improve the effectiveness of current immunotherapies by utilizing the properties of these microbial metabolites.

4. Therapeutic Strategies Combining the gut Microbiota With Immunotherapy

4.1 Fecal Microbiota Transplantation (FMT)

FMT is the process of transferring fecal microbiota from a healthy donor to the patient's gastrointestinal tract to restore the balance of the microbiota. Patients with recurrent Clostridium difficile infection (CDI) have responded well to FMT treatment [21], and it can alleviate symptoms of advanced liver disease. The ability of FMT to reconstruct the healthy intestinal flora and modulate the immune system in the host is central to the development of immunotherapy.

Studies have demonstrated that immune checkpoint inhibitors (ICIs), including anti-PD-1, can have a substantial impact on the effectiveness of gut flora composition. Melanoma is often resistant to anti-PD-1 therapy due to its specificity. Experiments by Gopalakrishnan et al. in mouse models showed that fecal microbiota transplantation can increase the sensitivity of solid tumors to immune checkpoint inhibitors (ICIs), thereby promoting tumor-specific CD8+ T cell responses and restoring anti-PD-1 therapy's therapeutic effect in melanoma patients [2]. Similarly, the report of Baruch et al. verified in human clinical trials that FMT treatment significantly increased CD8+ T cell activation while reducing interleukin–8–expressing myeloid cells [22]. FMT treatment improves patient survival by converting non-responders (patients who show no response to ICIs) into responders, which makes FMT a promising new approach to cancer treatment.

4.2 Probiotics

Probiotics are live microorganisms that enhance immunotherapy by modifying the intestinal flora and providing health advantages to the host. Probiotics are usually composed of specific strains with health-promoting properties, which are used in cancer treatment because of their capacity to reduce inflammation and regulate immune responses.

Probiotics can reduce the possibility of central nervous system (CNS) poisoning in HIV-1-positive individuals by downregulating IDO mRNA expression through connections to immune system cells located in gut-associated lymphoid tissue (GALT) [23]. It has been demonstrated that some probiotic strains, including Lactobacillus and Bifidobacterium, increase the effectiveness of ICIs by promoting dendritic cell maturation and enhancement, which modulate the intestinal flora and promote a more favorable immune environment. For example, Bifidobacterium has been reported to enhance the infiltration of CD8+ T lymphocytes into tumors and activates dendritic cells to improve anticancer activity [3,6].

In addition, probiotics can modulate the intestinal state and prevent dysbiosis. It works as a combination to help alleviate some gastrointestinal side effects of immunotherapy, such as diarrhea and colitis. The safety and oral availability of probiotics make them an attractive option for combination therapy with immunotherapy.

4.3 Engineered Microbiomes

The concept of engineering microbiome involves the deliberate manipulation of intestinal microorganisms or their metabolites to enhance therapeutic effects. One of the strategies is to stimulate macrophages with nanomedicines to promote M1 macrophage polarization and convert M2 macrophages into M1.

TAMs have the ability to promote new blood vessel formation, while destroying existing blood vessels, which causes tumor vascular leakage and promoting tumor development. The studies have shown that nanomedicines can use the tumor vascular leakage of TAMs to

enhance their specificity of movement within tumors [24]. Nanoparticles made of biomimetic materials can use cell membrane camouflage to improve their targeting and penetration capabilities. In particular technologies such as albumin biomimetic nanocorona [25] and M2 macrophage binding peptide (M2pep)-modified gelatin (GM) modified TAM targeting shells [26] have been shown to enable nanomedicines to be preferentially internalized by TAMs in tumors. In addition, M2-like TAM dual-targeting nanoparticles (M2NPs), a technique created by Qian et al., can decrease the size of mice's tumors and extend their survival by blocking the survival signal of M2-like TAM and reducing the expression of immune cytokines [27].

Given the tumor penetration ability and targeting specificity of nanomedicines, the special design of the morphology and targeting ligands of nanomedicines can enable them to target specific cells or tissues to repolarize TAMs into M1 macrophages and regulate the balance of macrophages in the tumor area. This technology provides an important platform for treating tumors, which are difficult to penetrate cells, and enhancing highly targeted immunotherapy.

5. Conclusion

Immunotherapy use of the gut microbiota represents a promising frontier in cancer treatment. This complex and dynamic community of microorganisms has been shown to significantly influence the ability of immune systems to recognize and fight tumors. As research continues to uncover the complex connection between gut microbiota and immune responses, modulation of the microbiota has been demonstrated to increase the effectiveness of immunotherapy, particularly in patients who were previously resistant to treatment. Key findings highlighted in this article include the potential of the gut microbiota to affect innate and adaptive immunity, primarily through activation of dendritic cells, polarization of macrophages, and enhancement of CD8+ T cell responses. These effects are mediated by specific microbial species, such as Bifidobacterium and Lactobacillus, and their metabolites, which play a key role in shaping the tumor microenvironment and influencing the success of immunotherapy. Manipulation of the gut microbiome through approaches such as FMT, probiotics, and engineered microbiomes has great therapeutic potential. FMT has demonstrated the ability to convert non-responders of immunotherapy to responders, while probiotics offer a less invasive approach to enhance immune responses and mitigate side effects. In addition, engineering the microbiome, especially through nanomedicine, provides a new way to precisely target tumor-associated immune cells and improve treatment outcomes. Although the gut microbiota is important for immunotherapy, there are still major gaps in efficacy due to individual differences in each patient. Future research may consider combining gene editing technologies such as CRISPR to achieve more personalized and effective cancer treatments and prevent negative patient reactions caused by individual microbiome differences. It follows that there is obviously a lot of untapped potential in this area that needs to be explored further.

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