Gut microbiota and cancer immunotherapy: mechanisms and modifications

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Abstract. Cancer is a global health issue, and the incidence and death rates are relatively high. In the aspect of cancer treatment, immunotherapy including immune checkpoint inhibitors and CAR-T cell therapy appear to be promising with less side effects compared to traditional methods such as chemotherapy and radiotherapy. However, the relatively low remission rate drives scientists to investigate the factors influencing the response to immunotherapy. A variety of microbes including bacteria, viruses and yeast colonize the gut, forming the gut microbiota. The gut microbiota play significant roles in infection diseases and autoimmune diseases because of the interplay between the intestinal microbiota and local mucosal immune system. Moreover, it has been found that there is a link between cancer treatment outcome and gut microbiota, indicating the great potential of modifying the gut microbiota as a treatment for boosting the therapy response rate. Unfortunately, the relationship between them remains unclear. Therefore, it is in an urgent need to further explore the mechanisms and functions of gut microbes in cancer immunity. This review introduces the impacts of gut microbiota on innate and adaptive immunity, the associations between therapy outcome and gut microbes, and discusses the modulation methods such as diet, fecal microbiota transplantation and probiotics.

Keywords: Cancer; immunotherapy; immunity.

1. Introduction

Cancer is a worldwide issue, a major cause of premature death, and a contributor to the loss of productivity. In 2023, the incidence rate (1,958,310 cases) and death rate (609,820 deaths) are relatively high in the United States. Cancer treatment places a large burden on global healthcare systems. The traditional treatment for cancer includes radiotherapy and chemotherapy. Accordingly, the pharmaceutical industry spends more than $6.5 billion on the development of cancer drugs every year. In recent years, much research on cancer and immunity have emerged. Accordingly, although immune cells are patrolling for abnormalities in human body, tumor cells develop mechanisms to escape immunosurveillance including expressing programmed death-ligand (PD-L1) and cytotoxic T lymphocyte-associated antigen-4 (CTLA-4). These molecules act as immune checkpoints. When binding to their receptors or ligands, it causes T cell inactivation [1]. In this context, immune checkpoint inhibitors such as anti-PD-1 and anti-CTLA-4 monoclonal antibodies have been introduced as cancer immunotherapy. However, the response rates are relatively low (approximately 20-25% in renal-cell cancer, non-small-cell lung cancer, and melanoma) [2]. Recent studies show that gut microbiota play important roles in maintaining the immune homeostasis and demonstrate its capacity of improving the response rate of chemotherapy and radiotherapy in cancer treatment, which implies the possibility of utilizing gut microbiota in cancer immunotherapy.

The gut microbiota contain approximately 40 trillion microorganisms [3]. It is important in regulating host metabolism and guiding development and function of the host immune system. In addition, the host immune system and the gut microbiota interact and influence each other to protect the host from infection and to maintain immune homeostasis [4]. The gut microbiota might contribute to tumor development. The microbe express antigens (pathogen-associated molecular patterns, PAMPs) that can be recognized by pattern recognition receptor (PRR). Prolonged PRR signaling indicates chronic inflammation and results in tissue damage which might promote tumorigenesis [4]. Although the tumor-promoting capacity is innegligible, gut microbiota is also associated with anti-

tumor immunity and response to therapy. For example, molecular mimicry between tumor cells and commensal bacteria contributes to better responses to immune checkpoint inhibitors by indirectly increase the immunogenicity of tumor cells [5]. The gut microbiota or its products can mimic tumor antigens, triggering systemic innate immune responses via PRR and causing T cell priming [6]. Gut microbiota not only impact the efficacy of cancer immunotherapy, but also influence the side effects or toxicity of the treatment. Results by Jain et al. indicate that the Ruminococcaceae family may promote immunosuppression, which is in line with claims in the literature that this family reduces colon inflammation and has fewer side effects when receiving immunotherapy [7]. Meanwhile, the Ruminococcaceae family is also found in responders to immunotherapy in patients with melanoma, which indicates that the gut microbiota can stimulate immune response against tumor development and limit side effects by immunosuppression at the same time. Since microbiome dysbiosis can cause detrimental effects, gut microbiota modulation (such as diet, fecal microbiota transplantation and probiotics) might be an alternative approach to enhance the efficacy of cancer immunotherapy and reduce the associated adverse effects.

The bacteria taxa that are associated with cancer immunotherapy vary from different research and limited research discuss on the mechanisms of microbiota influencing therapy response. This review summarizes the associations between gut microbiota and immunity and how cancer immunotherapy responses links with the composition of gut microbiota. At the end of this article, methods of modulating gut microbiota and future suggestion on the related research are provided.

2. Gut microbiota and immunity

According to Singh et al., pattern recognition receptor (PRR) ligands or metabolites produced by the gut microbiota could modify both innate immunity and adaptive immune response locally and systemically [8].

The two primary players in innate immunity against cancer are pro-inflammatory M1 macrophages and natural killer (NK) cells. The two primary players in innate immunity against cancer are pro-inflammatory M1 macrophages and natural killer (NK) cells. Lactobacillus plantarum can efficiently boosts the production of natural cytotoxicity receptor (NCR) proteins, activating NK cells and initiating innate response. While intratumoral microbiota ablation in pancreatic ductal adenocarcinoma may shift M2 macrophages towards M1 macrophages which encourage inflammation, Bifidobacterium may stimulate NK cells to fight against malignancies. When combined, they make tumors more susceptible to immunotherapy [1]. Besides, Bacteroides fragilis promotes innate immunity by inducing M1 macrophage phenotypic polarization and upregulating CD80 and CD86 cellular expression.

Regarding to adaptive immunity, bacteria like Bifidobacterium, Burkholderiales, and Bacteroides may improve DC-mediated anticancer T cell immunity for the potentiation of immunotherapy. Moreover, segment filamentous bacteria (SFB) can stimulate Th17 cells production in mice's lamina propria. Transplantating SFB in mice models induces the enhancement of CD4+T helper cells, and raises the levels of IL-17 and IL-22 [2]. In tumor tissues, Bifidobacterium, Enterococcus, faecalibacterium, Ruminococcus, and Clostridium stimulate the infiltration of CD8+ T lymphocytes.

Microbial products interact with PRR and cause mDCs to present cross-antigens to CD8+ T cells as well as producing cytokines, such as IL12 and CXCL9/10 [9]. Butyrate, a metabolite product, increases granzyme B and IFN-γ expression in CD8+ T cells. Meanwhile, butyrate exposure could induce the transition from effector CD8+ T cell to memory cells [4]. In addition, butyrate can influence cytokine production from DCs and affect Th17 cells activation. As a type of short-chain fatty acid (SCFA), butyrate also regulates metabolic pathways such as TCA cycle, glycolysis and b-oxidation. These processes provide energy to B cells as well as effector and memory T cells. However, another report proposes that SCFAs like butyrate can stimulate regulatory T cells (Tregs) differrentialtion, which secrete IL-10 creating an anti-inflammatory microenvironment and suppressing activities of multiple immune cells [10]. This distinction illustrates the complexity of

microbiome's impact on human immunity and demonstrates both pro-inflammatory and anti-inflammatory effect of the gut microbiota. Therefore, further studies may explore on the impacts of SCFAs.

3. Gut microbiota and cancer immunotherapy

3.1. Immune checkpoint inhibitor

The immunological checkpoint protein receptor known as CTLA-4 is overexpressed on Tregs. In tumor-bearing mice and melanoma patients, antibodies against CTLA4 partially reverse the immunosuppressive effects of tumors and provide long-lasting remissions. Cytotoxic T-lymphocytes (CTLs) express PD-1, which binds to the PD-L1 expressed by tumor and stromal cells. Also, this protein pair serves as an immunological checkpoint. Antibodies against PD-1 and PD-L1 stimulate CTL activity and prevent T-lymphocyte senescence. Summarily, both anti-CTLA4 and anti-PD1 (or anti-PDL1) mAbs function by inhibiting certain inhibitory signals that impede effector T cell antitumor responses. Although the immune checkpoint inhibitors (ICIs) are promising in cancer immunotherapy, the response rate is relatively low in 15-20% which needs improvements. Studies of stools from patients that receive ICIs treatment show that, compared to non-responders, responders show greater alpha diversity of bacteria in their gut. It implies that alpha diversity might be linked to response rate. Within patients with B cell malignancies, a low alpha diversity is associated to reduced probability of complete remission [11]. Additionally, it was discovered that patients' progression free survival (PFS) was considerably longer in those with higher alpha diversity in their stool. These results spark the investigations on the relations between gut microbiota and ICI response.

In murine studies, numerous taxa, such as , Faecalibacterium, Akkermansia, Bacteroides and Clostridiales spp., have been proposed to augment checkpoint blockade's overall anticancer efficacy [12]. In addition, Firmicutes (e.g. Lachnospiraceae spp., Ruminococcaceae spp.) and Actinobacteria (Coriobacteriacaea spp. and Bifidobacteriaceae spp.) are associated with favorable response to anti-PD-1 mAbs in melanoma patients by augmenting CD56+CD8+ T cell activation in ICI responders' peripheral blood [11,13]. What's more, mAbs that target CTLA-4 increase the numbers of the B. thetaiotaomicron, B. fragilis and Burkholderia cepacia growing in the intestinal mucosa. Polysaccharides produced by these bacteria stimulate DCs maturation, improving IL-12-dependent Th1 immune response in the tumor site [1,5,14]. These indicate that cancer immunotherapy and gut microbiota interact with each other. The therapy affects the composition of gut microbiota and the microbe influence the response rate at the same time.

The bacteria mainly affect the adaptive immunity. In peripheral blood, faecaliberium boosts the percentage of CD4+ T cells while lowering the percentage of Tregs [5]. Bifidobacterium can enhance therapeutic effects by increasing the number and maturation of DCs, increasing expression of major histocompatibility complex (MHC), and cytotoxic T lymphocytes activation [3,14]. Specifically, Bacteroides fragilis and Bifidobacterium pseudolongum induced a Th1 immune response, improving the effectiveness of ICIs. Bifidobacterium breve and Bifidobacterium longum are considered to associate with better anti-cancer responses. Bifidobacterium breve activates DCs for CD8+ T cell priming and infiltration while Bifidobacterium longum reduces PD-1 molecular expression and secretes hippurate which activate NK cells [1,14]. What's more, Bifidobacterium bifidum activates DCs, which improves tumour-specific CD8+T cell responses and restores anti-PD-L1 treatment efficacy [5].

Positive effect on immunotherapy has also been shown to relate the metabolite inosine produced by Akkermansia muciniphila and Bifidobacterium pseudolongum. When using anti-CTLA-4 mAbs, the intestinal barrier is impaired, which allows the metabolite inosine to reach the systemic circulation and activate Th1 cells, therefore enhancing tumor regression [1,3]. Inosine also boosts tumor cells' ability to display antigens, allowing cytotoxic immume cells to detect and eradicate tumor cells [5].

3.2. CAR-T cell therapy

Chimeric antigen receptor (CAR) T cell therapy has shown remarkable antitumor effectiveness in the treatment of hematological malignancies, making it a very promising cancer treatment. The Food and Drug Administration (FDA) has approved four CAR-T cell therapy to treat CD19-positive hematologic malignancies. Nevertheless, up to 80% of patients may develop CAR-mediated toxicities, such as cytokine release syndrome and immune effector cell-associated neurotoxicity syndrome. Besides, up to 60 percent of patients might have disease recrudescency [15]. Gut microbiota is a great contributor. It is shown that there is a strong connection between the response rate and microbe composition in the gut. The stool samples collected before treatment from those attain complete remission (CR) revealed a high abundance of Lachnospiraceae, Ruminococcaceae, and Scilliospiraceae [8,11]. In the meantime, Peptostreptococcaceae enrichment was seen in individuals who failed to achieve CR [8].

In a CAR-T cell therapy study, broad-spectrum antibiotics including piperacillin/tazobactam, meropenem, imipenem/cilastatin (PIM) were administered to 20.6% of the cohort [12]. It is found that PIM exposure prior the immunotherapy correlates to worse progression-free survival (PFS) and overall survival (OS). It is also reported that increased neurotoxicity is associated with PIM exposure in the four weeks before treatment [15]. Indeed, PIM exposure enhanced the risk of immune effector cell associated neurotoxicity in patients with non-Hodgkin lymphoma or B-cell acute lymphoblastic lymphoma [8]. The reason might be the use of antibiotics reduces taxa of Bacteroides, and Faecalibacterium in the gut microbiota which are correlated to adaptive immunity. According to Hu et al., taxonomic analysis of the fecal microbiome using 16S sequencing revealed that members such as Faecalibacterium, Bacteroides and Ruminococcus were related with positive outcome of CD19 CAR-T cell treatment [16]. It is found that, among multiple myeloma patients in complete remission versus those in partial remission, there are differences in the diversity and quantity of Prevotella, Collinsella, Bifidobacterium, and Sutteralla [16]. It is further shown that Day 100 complete response was linked to a relatively higher abundance of class Clostridia, which includes the genera Faecalibacterium and Ruminococcus, the species Faecalibacterium prausnitzii, the family Ruminococcaceae, and Ruminococcusbromii [15,16]. This is because high abundance of Faecalibacterium and genus Ruminococcushave is linked to increased neutrophils, monocytes and lymphocytes [8]. Several species are associated with no toxicity in CAR-T cell therapy. In addition to triggering a complete response, it shows that class Clostridia as well as species Feacalibacterium prausnitzii are related to no toxicity. Furthermore, microbes such as Blautia, Ruminococcus, Bacteroides, and Faecalibacterium have been associated with no toxicity [15].

4. Gut microbiota modulation

4.1. Diet

The researchers propose that modifications in a wide range of nutrients, such as protein, fats, fibre and carbohydrates, cause notable alterations in the microbiome of the human gut. It is assumed that diet manipulations could reconstitute the gut microbiota composition. Szczyrek et al. suggest that consuming protein from whey and peas raises Bifidobacterium and Lactobacillus levels [14]. Additionally, it inhibits the growth of Clostridium perfringens and Bacteroides fragilis. Furthermore, intestine SCFA levels are raised by pea protein. It is reported that high-fat diet potentially causes certain Clostridium species enrichment in the murine gut [4]. At the same time, it reduces Bifidobacterium spp. and Bacteroides while increases quantities of pro-inflammatory gut microbes [10]. The enterotypes dominated by Bacteroides and Bifidobacteres are inversely correlated with fiber consumption and favorably correlated with high intake of animal proteins or a high-fat diet [14]. Firmicutes and Proteobacteria, which are generally reduced in patients who consume a high-fat diet, are prompted by high fiber intake. In mice models, a high-fibre diet also expands the number of fibre-fermenting Ruminococcaceae spp. that induce T cell activation of infiltration to the tumor site [11].

Among 46 patients who got anti-PD-1 therapy, researchers discovered that those who maintained a high-fiber diet were around five times more susceptible to the immunotherapy than those who followed a low-fiber diet [17]. Low-fiber and high-fat diet is associated with colorectal carcinogenesis and tumor immune evasion with significant increased F. nucleatum. High polyunsaturated fat intake promotes Ruminococcus development in the gastrointestinal tract. Bacteroides grows rapidly with a diet high in simple sugars and carbohydrates. A salt injection causes decrease in Lactobacillus murinus levels in rat models while Th17 levels rise. Another study using rat models shown that a high-salt diet causes an increase in Firmicutes and a decrease in Bacteroides and Proteobacteria [14].

There are different types of diets, including Mediterranean diet, Western diet, and ketogenic diet. Mediterranean diet (consumption of plant-based fiber-rich food and healthy fats) increases the abundances of Clostridia and Bacteroidetes and decreases the prevalence of Firmicutes and Proteobacteria. It also increases the production of SCFAs, including butyrate, propionate and acetate, which produces Bacteroidetes and limits Firmicutes development [5,14]. According to Wang and Geng, shown in animal models, Western diet (described as a low intake of fiber, vegetables, and fruits and a high consumption of ultra-processed foods, fat, sugar, alcohol, and refined carbohydrates) result in increased levels of both Firmicute and Proteobacteria and devreased levels of Bacteroidetes [5]. It also claims a tenfold rise in the proportion of Bacteroides to Prevotella when treated with a Western diet. Bacteroides growth is linked to both the ketogenic diet (low carb and high-fat) and the animal-based diet. The fact that some Bacteroides species could enhance patients' reaction to anti-CTLA-4 therapy suggests that a ketogenic or animal-based diet may improve immunotherapy effectiveness.

Although diet can alter or reset the gut microbiota, it is not a long-lasting approach.

4.2. Fecal microbiota transplantation

Fecal microbiota transplantation (FMT) is another approach to manipulate the gut microbiome. It is noted that, compared to diet interventions, the effects of FMT could remain for more than six months, reducing the need for repeated interventions [1]. FMT is the most direct technique of changing the microbiota, in which a recipient receives stool from a donor through oral administration of frozen or lyophilized tablets, or direct delivery via gastroscopy or colonoscopy [10]. Studies of FMT combining with ICIs report positive outcome in patients with melanoma, prostate cancer, gastrointestinal by activating host immunity [10]. Shown in preclinical studies, FMT from human cancer patients into germ free mice could reproduce the phenotype of responder/non-responder [4]. In a study, germ-free mice that received FMT from PD-1 blockade responders showed improved anti-tumor immunity and were responsive to anti-PD-1 therapy. Meanwhile, germ-free mice given with stool from non-responders were unable to successfully generate a response to PD-1 inhibitors [10]. According to Davar et al., in a subgroup of patients with advanced melanoma, FMT used in combination with PD-1 inhibitors successfully colonized responders' guts and altered the tumor microenvironment, overcoming primary resistance to anti-PD-1 therapy [13]. To elicit clinical responses to anti-PD-1 mAbs in PD-1-refractory melanoma patients, FMT altered the composition of the microbiota towards taxa that supported treatment efficacy.

However, the downsides of FMT include transfer of bacteriophage, parasites, pathogenic bacteria, multidrug-resistance bacteria or bacterial associated with inflammation-induced carcinogenesis [4,14]. The method to bypass the risk of transferring pathogens using FMT is using bacterial isolates and consortia, which act for different patients consistently. Robust microbiological screening is also considered to be essential for abrogating the risk of transferring pathogens. Studies using FMT also have limitations, due to differences in commensal colonization ability in the gut and the depletion of strictly anaerobic bacteria following the transplant [11].

4.3. Antibiotics, prebiotics, probiotics

Antibiotics for treating secondary infections should be carefully used as it might cause gut microbiota dysbiosis and adversely affect the effectiveness of immunotherapies. Daver et al. found that using antibiotics for soft-tissue infections depleted beneficial taxz such as Ruminococcaceae spp.,

Alistipes spp., and Faecalibacterium prausnitzii, which had deleterious effects on anti-PD-1 in cancer patients [13]. In addition, Schett et al. found that patients with non-small cell lung cancer who received antibiotics within 60 days of ICI commencement had drastically lower PFS and OS while some studies reported that antibiotics administered 60 days before ICI therapy are strongly associated with shorter OS [18]. However, eliminating some bacteria with antibiotics can prevent the growth of tumors in the liver and colon in animals. For example, use of vancomycin inhibits primary and metastatic liver cancer growth in rats by stimulating NK cell migration of IFNγ production in the liver. This indicates that gut microbiota can be pro-carcinogenic or pro-inflammatory.

Prebiotics may aid in the restoration of gut microbial diversity and support cancer treatment. Prebiotics are specific non-digestible food ingredients, such as oligomers of fructose and galactose and inulin, that promote the growth of selective beneficial microbes and their metabolites including inosine (metabolite produced by Bifidobacterium pseudolongum) and SCFAs. SCFAs can reduce the pH of the colon, allowing beneficial microorganisms like Bifidobacterium and Lactobacillus to thrive [10]. Moreover, resistant starch promotes the growth of microorganisms involved in butyrate synthesis, which may decrease the production of pro-inflammatory cytokines.

Probiotics are foods and health products that enhance the populations of good bacteria in the gut by introducing living microbes. They are intended to prevent and cure dysbiosis, which is characterized by an unbalance or deficiency of helpful microbes in your microbiome. It is also used to restore innate and adaptive immunity. Preclinical studies suggest that taking probiotics could improve anticancer immunity by lowering Treg levels while increasing CD4+ T cell differentiation, CD8+ T cell activation, and NK cell intratumoral infiltration [1]. The antitumor activity of CTLA-4 mAb was found to be enhanced in model mice by Lactobacillus acidophilus cell lysates. This was due to an increase in effector memory T cells and CD8+ T cells, a decrease in M2 macrophages and Treg cells in the tumor microenvironment, as well as a slight reversal of gut microbiota dysbiosis [5]. In the anti-PD-1 treatment context, the use of Bifidobacteria species has the potential to facilitate anti-tumor T cell responses and therefore boost the efficacy of the immunotherapy [19]. In addition, patients with refractory cancer can benefit greatly from probiotics and bacterial consortia that contain live bacteria, such as Propionibacterium, Bifidobacteria, Streptococcus thermophilus, and Lactobacillus, when paired with mAbs. Probiotics have been shown to decrease IL-8 positive myeloid cells and increase pro-inflammatory cytokines in patients [8]. Furthermore, researchers have discovered that the amount of Lactobacillus in the gut significantly decreased in patients with severe ICI-related colitis, and subsequent research has verified that this probiotic can alleviate ICI-related colitis through oral administration [3]. It is also suggested that to accurately inject probiotics into cancer therapeutic regimens, metagenomics investigation of the functional and taxonomic relationships between beneficial microorganisms and resident microbes is necessary [8].

5. Conclusion

The exploration of the association between gut microbiota and treatment response brings hope to improve the response rate of cancer immunotherapy. Altering gut microbiota composition, for example, increasing beneficial bacteria such as Bacteroids, faecalibacterium and Ruminococcus, might contribute to efficacy enhancement and no toxicity in both ICIs treatment and CAR-T cell therapy. There are different ways to modulate gut microbiota, including diet manipulations, FMT, antibiotics, prebiotics and probiotics. Firstly, a high-fibre, low-fat diet with simple sugar and carbohydrates might boost the sensitivity to cancer immunotherapy. Secondly, FMT is a long-lasting approach to change the composition of the gut microbiota that related to positive outcome and improved efficacy. Thirdly, some antibiotics can kill the pro-tumorigenesis bacteria to improve treatment outcome. Finally, prebiotics and probiotics can enhance the quantity of good bacteria in the gut and interact with the immune system to augment the efficacy of the therapy. However, FMT and antibiotics should be carefully administered. The reason is that FMT might introduce harmful pathogens while antibiotics might reduce the beneficial microbes.

In addition to the contradictory facts of SCFAs, there are some limitations of the research design. Firstly, the factors that influence gut microbiota include age, gender and cancer type, which might contribute to different results. Additionally, study designs differ in sample collection, treatment regiment, inclusion/exclusion criteria and data analysis, which might place challenge to discover repeatable and generalizable microbes that affect therapy efficacy and adverse effects [6,12]. It is suggested that artificial intelligence may be useful in study design. Secondly, in addition to bacteria, gut microbiota also consists of virus, fungi, and other microbial and eukaryotic species, which mainly located in the colon and small intestine. Most research only study the association between bacteria and therapy response, omitting the effect of fungi and virus. Moreover, the strategy of collecting feces sample for analysis only reveal the bacterial lying in the colon, excluding much that lies in the small intestine. Thirdly, other than gut microbiome imbalance, immunotherapy resistance might develop from tumor-intrinsic oncogenic pathways and tumor microenvironmental factors, which are out of consideration in the research. Finally, most of the research study the associations between immunotherapy response and gut microbiota, which do not indicate the causation effect of microbes. It lacks rationale to alter gut microbiota for better therapy response.

For future directions, feces sample before and after treatment should be collected to explore the interactions between gut microbiome and therapy response. Additionally, the mechanisms of microbe metabolites in cancer immunity need further investigations. Last but not least, using artificial intelligence and exploring microbial gene content might be useful in determining predictive biomarkers of therapy outcome.

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