

Role of Gut Microbe in Cancer Immunotherapy

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Abstract. Gut microbes have become more and more popular as their balance is closely related to human health and immunity. The gut has its own mucosal structure which helps prevent pathogenic bacteria from invasion and working with gut microbes to deal with diseases. The gut microbe can affect human immunity through its metabolite and its connection with the mucosal system. They can also help build barriers to prevent hosts from the pathogens. While the dysregulation of gut microbe will cause inflammation, diseases, and tumor growth. The growth of pathogens and the drop of probiotics destroy the function of the host's immunity and thus result in some gut diseases, such as autoimmune diseases and colorectal cancer. Also, clinical trials have shown that the gut microbe has a great effect on cancer immunotherapy, especially immune checkpoint inhibitors. Research has proved many kinds of bacteria in the human gut can help regulate and promote the blocking of common immune checkpoints like CTL-4. They can take part in cancer immunotherapy by their secretion to trigger the human immune system and help patients deal with tumors as well. Here the structure of the gut immune system, the common gut diseases, and the roles played by gut microbes in them are discussed. Also, the link between gut microbe and cancer immunotherapy will be included. Finally, the challenges and future development of the gut microbe in cancer treatment are briefly mentioned.

Keywords: Gut microbe; cancer immunotherapy; intestinal diseases; autoimmune diseases; mucosal immune.

1. Introduction

Microbe exists nearly everywhere in the human body. The human microbe includes many kinds of species, such as bacteria, archaea, protozoa, and so on. These microorganisms make up the human microbe and play a crucial part in many aspects. Among these, the gut microbe is considered to be one of the largest microbe groups and most of them are symbiotic with the host and the majority of them are bacteria, most of them are anaerobic. The gut microbe has a great connection with the host's diseases, inflammation, metabolism, and immunity [1]. In a healthy host's body, these microbes can help to build a wonderful microbial diversity in the gut, as well as prevent pathogen invasion and control the overgrowth of pathological organisms [2]. Its unbalance will result in dysfunction in the gut. For example, the ruin of the mucosal and entrance of pathogens, and thus induce diseases. Research shows that many kinds of diseases have a connection with gut microbes. Including diseases in many parts such as the nervous system, respiratory system, cardiovascular system, and of course, cancer [3].

As research shows, gut microbes have non-negligible impacts on tumor growth. The dysregulation of the gut microbe is increasingly associated with tumorigenesis. Imbalance or disorder of the gut microbes may be constituted by a variety of causes, such as poor dietary habits, various environmental factors, and even genetic factors. According to the results of the clinical trials, the intestinal flora of the patients had a very significant difference compared to healthy participants. The most obvious manifestation of this is a drop in probiotics and a boost in pathogens. These pathogenic bacteria cause cancer by releasing some toxins or specific metabolites that disrupt the normal function of the body's immune system, cause inflammatory responses, and suppress oncogenes [4]. For instance, the growth of *Helicobacter pylori* will induce degradation of the tumor inhibition gene p53, which contributes to the growth of the tumor. *Clostridium perfringens* produces a specific protein that functions as the immune receptor of T cells and NK cells thus blocking toxic effect of immune cells on tumor cells and forming malignant tumors [5].

Cancer immunotherapy has been considered a popular research target nowadays and already being a famous method against cancer. Gut microbes have been a hot topic of research in recent years, and their connection with cancer immunotherapy has received much attention as well [6]. Some probiotics have also been shown to promote cancer immunotherapy, such as triggering the immune cells to fight against tumors and enhancing the efficiency of immune checkpoint inhibitors(ICIs) [7].

This review will talk about the structure of the gut immune system, some common gut diseases caused by imbalanced microbes, and the relationship between gut microbes and cancer immunotherapy. Also, challenges and future perspectives of gut microbes in cancer immunotherapy will be briefly introduced.

2. The gut mucosal immune system

It is more familiar with the gut as the main place for digestion and absorption of nutrients, but the gut is also a crucial part of preventing pathogens and is the largest immune system of humans. The mucosal system is a critical component of the gut immune system [8]. The mucosal system differs from the other immune system because it is a highly differentiated immune system that is found mainly in some mucosal tissue sites, such as the skin, intestines, nasal mucosa, respiratory tract, oral cavity, bronchi, genitourinary tract, and so on. The gut mucosa is the most significant member because it has a much larger mucosal surface area than the other mucous membranes⁹. The gut mucosal surface area is huge, and the same with the outside world directly, and the intestinal tract of various organic and inorganic substances in direct contact, so the mucosal system is considered the first protective barrier against pathogens.

The mucosal of the gut is dominated by the lymphoid tissues associated with the gut, including Peyer's patches, lamina propria as well as intestinal epithelium [9]. Intestinal epithelium is very tightly connected to each other, and outside the intestinal epithelium, there is a mucus layer. The mucus layer has many kinds of proteins including antimicrobial peptides, defensins, and immune globulin such as IgA. Also, the host's gut microbe can live here. It is a wall between the contents of the intestine and the underlying tissues. When the intestinal epithelial cells are damaged, the hosts will be exposed to infection or injury, and intestinal permeability increases [10]. In lesser degrees of disruption, peripheral epithelial communities migrate to the site of injury and promote wound healing. In inflammatory bowel disease (IBD), where the injury is recurrent and injures the crypt structure, intestinal stem cells migrate to the wound and renew the intestinal epithelium [11].

Underneath the intestinal epithelium is the lamina propria of the gut. The lamina propria has many kinds of immune cells in it, such as T cells, plasma cells, intrinsic lymphoid cells, DCs, granulocytes, etc. The immune cells also exist in the intestinal mucosa. The large number of immune cells builds a strong barrier of immunity in the gut mucosa [9].

The Peyer's patches, a crucial part of the gut mucosal system, are a kind of lymphoid follicles within the mucosa of the small intestine. Lymphoid follicles are made up of plasma cells and T cells (CD4 predominantly) and over these cells is a layer of microscopic wrinkled cells which are called M cells. It recognizes the viruses and enteropathogenic bacteria that are swallowed and delivers these swallowed antigens in the intestinal lumen to the dendritic cells, the DCs can then process, and transport pathogenic antigens to activate immune cells. After that, these activated immune cells will go back to the gut mucosal lamina propria through the process of cyclic homing and become plasma B cells and effector T cells to take part in the fighting against the pathogens [12].

3. Gut microbe and diseases

3.1. Intestinal diseases

As the body grows and develops, the microorganisms in the gut are also constantly interacting with the mucosal surfaces in the gut. Therefore, gut microorganisms have become a significant member in protecting the human body. Comparatively, the balance and dysbiosis of the gut microbes

are also closely connected with the patient's level of health. If the gut microbes are dysregulated, it can cause a host of problems. For example, the intestinal mucosa is damaged, immunity is reduced, and inflammation is caused [11]. Many kinds of intestinal diseases are proven to have a great connection with gut microbes. For instance, inflammatory bowel disease (IBD) and Colorectal Cancer (CRC) [13].

IBD is considered a typical inflammatory bowel disease associated with deviations in the composition of gut microbes. It includes some different kinds of non-specific chronic gastrointestinal inflammatory diseases. These include ulcerative colitis (UC), Crohn disease (CD), and indeterminate colitis (IC). The high incidence of IBD is strongly connected with the imbalances or disorders of the host's gut microbe. Cesarean section, excessive disinfection, and overuse of antibiotics can all result in no access to the microbe's normal environment early in life, leading to a lack of negative regulatory pathways and an over-response to the normal gut microbe. Also, the dysregulated gut microbe will destroy those layers of protection we mentioned above, and thus cause inflammation and finally IBD [3].

Clinical trials have revealed that the gut microbial composition of participants with inflammatory bowel disease is altered, and the diversity of gut microbes is significantly reduced. These include a decrease in the abundance of Firmicutes, the disappearance of Clostridium cluster, and an increase in the abundance of Enterobacteriaceae. The imbalance of the patient's gut microbes leads to a drop in the secretion of short-chain fatty acids, amino acids, vitamins as well as IgA. These changes not only destroy the gut mucosal layer and bring about the invasion of pathogenic bacteria but also, more seriously, affect the normal function of the gut immune system, resulting in an inflammatory response that can lead to IBD [11].

Colorectal Cancer (CRC) is another common disease caused by gut microbes through microbes and their metabolites. CRC is multifactorial like other cancers, but it is a disease in which environmental factors outweigh other factors such as genetics. It is also a result of the breakdown of the protective effects described above, allowing the gut microbe to enter a state of imbalance and the intestinal barrier is disrupted, short-chain fatty acid levels are reduced, especially Butyrate. Butyrate, an anti-inflammatory molecule, inhibits histone deacetylase in immune cells, down-regulates cytokines which promote inflammation, and induces apoptosis in CRC cells [10]. Bile acids are also closely related metabolites. Excessive intake of secondary bile acids in people with high fat can lead to an increasing risk of CRC. Besides, deoxycholic acid has the ability to promote oxidative DNA damage in vitro and promote tumor formation in vivo. The imbalance of gut microbe can also cause the growth of some kinds of pathogenic bacteria which can produce an inflammation environment in the colon. For example, the *F. nucleatum* activates the nuclear factor κ B pathway, promotes myeloid infiltration in tumors, and creates a pro-inflammatory environment conducive to CRC progression in ApcMin mice (a common CRC model) [5].

3.2. Autoimmune diseases

Autoimmune diseases (AIDs) are caused by a change in the host's immunity, and there exists an attack on its tissues by the immune system. The diseases include systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). The complex pathogenesis of these diseases is still understudied. Although it has been suggested that autoimmune diseases may be genetically acquired, environmental factors have also received widespread attention for their contribution to disease initiation and transmission [3]. Rapid progress in high throughput sequencing technology raises the possibility of studying a multitude of gut microbes, and genomic studies of gastrointestinal disorders have found that individuals with the disease are different from healthy volunteers, indicating that the ecology of the gut microbe is out of balance [6].

A complete mucosal is needed to keep the balance between microbes and the host's immune system in order to prevent inducing over-response of the immune system. However, the intestinal wall of AIDs patients is usually associated with damage and leakage. Damage to the intestinal mucosal raises the translocation of intestinal microbes and other components, resulting in aberrant

exposure of gut microbes to the immune cells and arousing AIDs by different kinds of pathways. For instance, it has been detected that *Enterococcus gallinarum* showed up in the livers of patients with SLE, whereas it didn't appear in normal livers. Also, certain bacteria rich in intestinal and other related pathogens may come from the oral cavity, such as RA [14].

Molecular mimicry is a biological phenomenon in which certain molecular structures of a pathogen are similar to those of the host's molecules, leading the immune system to mistake the host's tissues for the pathogen, resulting in an autoimmune response. For example, bacteria such as *Bacteroides fragilis*, *Candida albicans*, and *Streptococcus sanguis* have peptides that are alike to type II collagen, thus causing autoimmune response diseases, while *Roseburia intestinalis*, *Bacteroides thetaiotaomicron*, and others can also cause lupus erythematosus-like symptoms [7].

An unbalanced gut microbe and its derivatives, including DNA or RNA, carbohydrates, enzymes, and vitamins, can lead to over-reaction of the host's immune system, resulting in diseases. Adaptive immunity is also a significant reason for autoimmune diseases, and those unbalanced bacteria and their metabolites with proinflammatory capacity may aberrantly activate the adaptive immune system through innate immune hyperactivation, followed by triggering cell immunity: antigen-presenting cells can acquire pathogens and their metabolites from the gut to serve as antigens, and then send them to spleen or lymphatic nodes to activate T cells and plasma cells, and abnormal activation of immune cells can lead to autoimmune disease [11].

4. Gut microbe and cancer immunotherapy

As mentioned above, the gut microbe has a great connection to the host's immunity. Besides, it plays a significant role in cancer immunotherapy, especially in immune checkpoint inhibitors (ICIs) [4].

Immune checkpoints are a group of key factors expressed on immune cells that regulate the level of immune activation, and they keep the activation of the immune system within an appropriate range. But they are also utilized by some tumor cells to inhibit the immune response and escape the attack from the immune cells like effector T cells. Thus, the occurrence of ICIs is a huge breakthrough in cancer immunotherapy. ICIs can release this inhibition and allow the immune cells to reactivate their work and destroy the cancer cells. The commonly used targets of the ICIs include programmed cell death 1 (PD-1), lymphocyte-activating gene-3 (LAG3), and cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) [6].

Gut microbes along with the metabolites they make have a great impact on the regulation of gut and body's immune responses, and research into their role in cancer immunotherapy and ICIs has received increasing attention in recent years. Control of gut microbe can strengthen the ability to fight against tumors and the therapies by ICIs.

Gut microbe improves ICI response by modulating innate immunity, adaptive immunity, and immunogenicity of tumor cells [5]. In terms of innate immunity regulation, intestinal flora could affect the immune response of dendritic cells (DCs), monocyte macrophages, and NK cells. In terms of adaptive immunomodulation, intestinal flora could affect the capabilities of effector T cells and CD4⁺ T cells. Research has shown that taking *Bifidobacterium* can activate the DCs, which stimulate the effector T cells as well as improve the efficiency of PD-1 inhibition therapy. Also, taking the *Bacteroides fragilis* can enhance the dendritic cells as well as the TH1 cell response, in order to improve the efficiency of the CTLA-4 inhibition therapy [3].

Gut microbes can also mediate anti-tumor immune responses to ICIs through their metabolites. These metabolites are synthesized or transformed by the gut microbe and can diffuse from their original places and have the ability to trigger the host's immune responses, thus improving the efficacy of ICIs. For example, the short-chain fatty acids (SCFAs) [10]. Propionic acid made by *Mucinophilic Ackermannia* inhibits the tumor growth, leads to apoptosis, and enhances the ability of ICI by G protein-coupled receptor 43 (GPR43) and down-regulated apoptosis protein (IAP) inhibitor as well as stimulating cell cycle inhibitor p21. SCFAs can also send nutrition to plasma cells, memory

T cells, and CD8 T cells through manipulating several pathways. For example, the glycolysis and tricarboxylic acid cycle. Thus they can help to promote the therapeutic effect of ICIs [15].

5. Conclusion

The gut is an indispensable organ in the human body. There are more and more research has proved that the gut microbe has a close relationship with human immunity, including cancer immunotherapy. On the one hand, the imbalance of the gut microbe can regulate the host's mucosal system and cause several illnesses, like IBD, colorectal cancer, along with autoimmune diseases. On the other hand, gut microbe contributes to cancer immunotherapy by the ability to promote the efficiency of ICIs. Once it was found, ICIs have already become a breakthrough in treating solid tumors. The gut microbe can fight against cancer by modulating the host's innate and adaptive immunity.

All in all, the time for gut microbe has already arrived. Clinical studies on gut microbes in human immunity and disease have become more frequent, and no one questions the therapeutic promise of gut microbes in cancer immunity anymore. However, there are still many unknown aspects about how gut microbes specifically fight cancer and various immune diseases and inflammation. The efficacy of many gut microbes as treatments for various cancers is still unknown in many cases, and there are still numerous clinical trials to be done and challenges to be faced. Moreover, the use of oral intestinal microbes as therapeutic treatments has to take into account the impact on the patient's pre-existing microbiota. It is expected that in the future, more and more microbial therapies will be available and the relationship between gut microbes and cancer immunotherapy will become clearer as well.

References

- [1] Manos, J. The human microbiome in disease and pathology. *APMIS* 130, 690–705 (2022).
- [2] De Vos, W. M., Tilg, H., Van Hul, M. & Cani, P. D. Gut microbiome and health: mechanistic insights. *Gut* 71, 1020–1032 (2022).
- [3] Wiertsema, S. P., Van Bergenhenegouwen, J., Garssen, J. & Knippels, L. M. J. The Interplay between the Gut Microbiome and the Immune System in the Context of Infectious Diseases throughout Life and the Role of Nutrition in Optimizing Treatment Strategies. *Nutrients* 13, 886 (2021).
- [4] Sepich-Poore, G. D. et al. The microbiome and human cancer. *Science* 371, eabc4552 (2021).
- [5] Matson, V., Chervin, C. S. & Gajewski, T. F. Cancer and the Microbiome—Influence of the Commensal Microbiota on Cancer, Immune Responses, and Immunotherapy. *Gastroenterology* 160, 600–613 (2021).
- [6] Li, W., Deng, Y., Chu, Q. & Zhang, P. Gut microbiome and cancer immunotherapy. *Cancer Lett.* 447, 41–47 (2019).
- [7] Zhang, Y. & Zhang, Z. The history and advances in cancer immunotherapy: understanding the characteristics of tumor-infiltrating immune cells and their therapeutic implications. *Cell. Mol. Immunol.* 17, 807–821 (2020).
- [8] Li, Y., Jin, L. & Chen, T. The Effects of Secretory IgA in the Mucosal Immune System. *BioMed Res. Int.* 2020, 1–6 (2020).
- [9] Hasegawa, M. & Inohara, N. Regulation of the gut microbiota by the mucosal immune system in mice. *Int. Immunol.* 26, 481–487 (2014).
- [10] Coker, O. O. et al. Altered gut metabolites and microbiota interactions are implicated in colorectal carcinogenesis and can be non-invasive diagnostic biomarkers. *Microbiome* 10, 35 (2022).
- [11] Schirmer, M., Garner, A., Vlamakis, H. & Xavier, R. J. Microbial genes and pathways in inflammatory bowel disease. *Nat. Rev. Microbiol.* 17, 497–511 (2019).
- [12] Da Silva, C., Wagner, C., Bonnardel, J., Gorvel, J.-P. & Lelouard, H. The Peyer's Patch Mononuclear Phagocyte System at Steady State and during Infection. *Front. Immunol.* 8, 1254 (2017).
- [13] Ogino, T. & Takeda, K. Immunoregulation by antigen-presenting cells in human intestinal lamina propria. *Front. Immunol.* 14, 1138971 (2023).

- [14] Wang, M., Liu, Y., Zhao, L. & Zhang, X. Modulating gut microbiota in autoimmune diseases: a cutting-edge strategy from prophylaxis to therapeutics. *Sci. Bull.* 67, 771–773 (2022).
- [15] Lu, Y. et al. Gut microbiota influence immunotherapy responses: mechanisms and therapeutic strategies. *J. Hematol. Oncol.* 15, 47 (2022).