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The human microbiome and the tumor microenvironment

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Abstract

The human microbiome has emerged as an intriguing field of scientific research. Its role in human physiology impacts both health and disease, contributing to the enhancement or impairment of metabolic and immune functions. Sometimes referred to as our body's "second genome", the alteration of the microbiome's bacterial ecology (dysbiosis), is linked to increasing numbers of illnesses, including cancer. The tumor microenvironment (TME) is the environment in which tumors grow and modulate the tumorigenic process depending on a myriad of distinct factors, including cell types, vascular system, and cytokines. Given the emerging relationship between the microbiome and the TME, this perspective aims to distill some of the key factors regulating the crosstalk between the microbiome and the TME. It also outlines why manipulating the microbiome may be a feasible strategy for anti-cancer therapy.

Keywords

Microbiome, microbiota, tumor microenvironment, immunotherapy

Introduction

Tumorigenesis is a multistep and complex process involving both genetic and epigenetic changes within the tumor cell in addition to supportive conditions within the tumor microenvironment (TME). The TME is intimately involved in carcinogenesis as it contains cells that liaise with and influence surrounding cells. Moreover, nonmalignant cells in the TME also influence the development of cancer by modulating cell proliferation. As a result, the TME has become the focus of intense scrutiny given the myriad factors that contribute to tumor growth. In addition to the genomic and epigenomic abnormalities within tumor cells that trigger changes supporting the formation of a TME, it also comprises the cellular component (hematopoietic and nonhematopoietic), the non-cellular extracellular matrix (ECM), and the liquid milieu comprising cytokines, hormones, and growth factors) [1].

The human microbiome refers to bacteria, viruses, fungi, and protozoa have emerged as an important focus of scientific inquiry given the vital role it plays in influencing human health. The microbiome genome is often referred to as our second genome, comprising over 1,000 species totaling an estimated 100 trillion microbes. Several factors influence their composition, including inter-individual variation, diet, ancestry, and

geographic location. Since 70% of the immune system is located in the gut, the microbiome residing in the gastrointestinal tract confers many benefits to the host, particularly relating to immune homeostasis [2]. Several studies have shown that alterations of the gut microbiome can cause immune dysregulation resulting in immune disorders [3]. Moreover, it can also influence gene expression, so proper nurture is required to maintain the balance of this complex ecosystem [4].

The host immune system and the gut microbiota

It is well established that microbes residing in the human body affect a large number of physiological processes. The gut microbiota plays a fundamental role in the development and maintenance of the host immune system. Moreover, the diversity of the gut microbiota is critical for the establishment of immune regulation networks. There is a symbiotic relationship between the gut microbiota and the host immune system that promotes host homeostasis. Several diseases including metabolic, neoplastic, and psychiatric disorders are influenced by the microbiome composition [5].

The microbiome can influence both organs that are in direct contact with the microbiome and those that are distally located, referred to as microbial dysbiosis. Moreover, altered gut microbiota may alter the efficacy of chemotherapy and radiation therapy, highlighting the intricate connections with neoplastic diseases. Several studies over the last few years have reported that the intestinal microbiota composition has a profound impact on influencing the therapeutic efficacy of immunotherapies. This includes the efficacy of checkpoint inhibitor antibodies against cytotoxic T lymphocyte protein 4 (CTLA-4) or programmed cell death protein-1 (PD-1), and anticancer immunosurveillance [6]. Moreover, the involvement of the gut microbiota in the metabolism of TME also affects the efficacy of conventional chemotherapy in combination with immunotherapy for pancreatic cancer [7]. The altered gut microbiota may alter the efficacy of chemotherapy and radiation therapy, highlighting the intricate connections with neoplastic diseases. Based on this complicated liaison between the host microbiome and immune response in the TME, manipulating the gut microbiota is deemed a feasible strategy for anticancer therapy [8].

The TME and the microbiome

The TME refers to the cellular environment whereby cancer stem cells, the cells within a tumor, have the ability to self-renew, thus driving tumorigenesis. Moreover, the TME is composed of a myriad of different cell types that include bone marrow-derived inflammatory cells, immune cells, blood vessels, lymphocytes, fibroblasts, signaling molecules, and the ECM [9]. Cancer development and progression are also influenced by the crosstalk between both malignant and nonmalignant cells within the TME, whereby the nonmalignant cells often play a pro-tumorigenic role at all stages of carcinogenesis [10].

Some of the key players in the immune system are also affected by the microbiota. For example, dendritic cells are one of the first immune cells to encounter microbes [11]. They have been shown to potentiate the anti-tumor effects of cluster of differentiation 8 (CD8) T cells following lympho-ablation with radiotherapy in melanoma murine models, and both interleukin-1 (IL-1) and IL-12 exposure to gut commensals by $CD103^+$ dendritic cells (DCs) is also critical to induce activated cytotoxic T cell responses that mediate the immunomodulatory effects of chemotherapy in addition to immunotherapy in several tumor models [12]. Tumor-associated macrophages (TAMs), are comprised of both M1 and M2 phenotypes. M2-type macrophages generate both cytokines and chemokines in the TME that diminish cytotoxic T cell responses and promote tumor growth and metastasis. It has been shown that microbial dysbiosis induces M2 phenotype and creates an immunosuppressive environment thus promoting growth of colon cancer [13]. Induction of both TAMs and myeloid-derived-suppressor cells (MDSCs) by Fusobacterium in the TME has been shown to suppress T cell responses and accelerate colorectal cancer (CRC) progression [14].

The composition of the gut microbiota includes microbes, such as bacteria, fungi, viruses, and mycoplasma, that also reside within tumor tissues. The low biomass of the tumor microbiome, first detected in humans over 100 years ago, has led to challenges in the characterization of its component parts. Recently, however, a comprehensive study of the tumor microbiome reported that an increasing number of tumors harbor bacteria,

suggesting the microbiome may be a vital component of the complex tumor ecosystem [15]. In this study, 1,526 samples and corresponding adjacent normal tissue across seven tumor types (including lung, breast, pancreatic, melanoma, and ovary) were investigated and the tumor type had a distinct tumor composition. The detection rate of bacteria varied between 14.3% in melanoma to > 60% in breast, pancreatic, and bone. Of particular interest, the bacteria were found to be predominately located in the intracellular cytoplasm of both immune and tumor cells.

Mechanisms of action

Several studies have investigated the mechanism of action underlying role of the tumor microbiome in the TME, see review by Ma et al. (2021) [16]. These mechanisms include:

- Cancer and immune cell presentation of bacterial peptides.
- Tumor antigens and mimicry by bacterial antigens.
- Immunogenic cell death induced by microbes.
- Pattern recognition receptor-mediated signaling pathway and adjuvants.
- Microbe-derived metabolites.
- Stimulation of inhibitory checkpoints.

Since these bacteria are metabolically active, the question arises whether these intracellular bacteria influence biological processes through their autonomous metabolism? Also, which molecules mediate these effects, or, alternatively, does the microbiome shape the TME via a non-cell-autonomous mechanism? The answers to these questions have important implications for the development of microbiome-targeted treatments that could potentially improve the efficacies of current cancer treatments [17].

Crosstalk within the TME

There is a long-established link between human cancers and viruses, such as human papillomavirus and oral, genital, and cervical cancers; hepatitis C and cervical cancer, Epstein-Barr virus and lymphomas [18]. Multiple studies have reported individual microbial species are involved in the progression of several cancers [19]. Some of the most well-characterized include Helicobacter pylori for both gastric cancer and mucosa-associated lymphoid tissue lymphoma (MALT lymphoma), *Streptococcus Bovis* for colon cancer, *Chlamydia pneumoniae* for lung cancer, and Salmonella typhi for gallbladder cancer. Moreover, lung cancers with tumor protein p53 (TP53) mutations have been associated with a large abundance of *Acidovorax temperans*. Other studies have also focused on the microbiota in cancers at distal sites and how it can be used to predict a successful response to cancer therapy in addition to enhancing the efficacy of existing therapeutics.

The interactions and crosstalk between the microbiota and TME are a two-way street whereby the cancer microenvironment itself can enhance the procarcinogenic activities of the microbiota [20]. Microbes within the TME can induce a mix of direct and indirect effects to impact tumorigenesis. From prior work largely on CRC, it is known that bacteria within tumors can cause chronic inflammation or produce and release toxins that impact the cell cycle and induce DNA damage that leads to tumor-initiating or -promoting mutations [21]. Microbes in the TME can also influence tissue remodeling and deregulate mucosal immunity, creating a favorable niche for tumor cells to expand and migrate [22]. Moreover, bacteria can induce epigenetic alterations upon gaining intracellular access that can activate dormant tumor-promoting genes [23].

It has also been reported that Fusobacteria can travel intracellularly within a migrating host colorectal cell and infiltrate distal sites such as the liver [24], a process mediated by the cytokines IL-8 and C-X-C motif chemokine ligand 1 (CXCL1) are specifically secreted upon *Fusobacterium nucleatum* (*F. nucleatum*) invasion of HCT116 CRC cells. This bacterium induced alterations at the epigenomic level whereby *F. nucleatum* infection, in conjunction with *Hungatella hathewayi*, induced the hypermethylation of tumor suppressor gene promoters in colonic epithelial tissue [25].

Microbial metabolites and the TME

In addition to modulating the host biology and TME via direct cell interaction, microbial-derived metabolites are also involved in this process. Two of the principal components include polyamine metabolites and short-chain fatty acids [26]. Polyamines are involved in several cellular processes including proliferation, apoptosis, and signal transduction. Those that are involved in epithelial turnover and gut barrier function include putrescine, spermidine, and spermine. Increased polyamine levels have been observed in CRC and reportedly enhance tumor development [27]. Short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate are generated in the colon and are essential for intestinal homeostasis, and have wide-ranging impacts on host physiology. Butyrate has a number of immune-modulating effects that include inhibiting lipopolysaccharide (LPS)-induced proinflammatory cytokine expression in dendritic cells, enhancing M2-macrophage polarization, and increasing T-regs thus influencing the TME and tumor progression recent study has shown that SCFA concentrations may be associated with PD-1 efficacy [28].

Cancer immunotherapy and the microbiome

Cancer immunotherapy spans a wide spectrum of immune modulation to enhance the immune system's ability to fight cancer. Of all the strategies employed to date, suppressing the negative immune regulatory factors, such as anti-PD-1, programmed death ligand-1 (PDL-1), and CTLA-4 by monoclonal antibodies has received the most attention. It has been reported that the response or inactivity of cancer patients to immunotherapy is dependent on the intestinal flora [2]. Moreover, the clinical response to checkpoint inhibitors varies among patients, and this variance may be attributable to the gut microbiome.

In spite of impressive clinical outcomes, the efficacy of immune checkpoint inhibitor (ICI) therapy remains variable. Some studies have provided compelling evidence that the patients' gut microbiotas affect their responsiveness to ICIs [29]. There are two phyla that dominate the human gut microbiota, *Firmicutes* and *Bacteroidetes*, and no strains are conserved universally among individuals. Therefore, the variations in clinical outcomes reported for ICI therapy may be attributable to differences in the gut microbiota within a given population.

There is an increasing focus on optimizing the microbiota composition to improve immunotherapeutic outcomes. Results of preclinical models have shown some promise, with further studies translating these observations into a clinical setting. For example, significant and accelerated tumor progression was observed in xenograft mice receiving ICI therapy that had been cotreated with antibiotics. When translated to a clinical setting, a diminished response rate was reported in those patients that were either pretreated or treated during ICI therapy with antibiotics, relative to controls [30]. In this study, two specific bacterial species were identified as being particularly predictive of ICI responsiveness that was largely due to increased infiltration of specific CD4 helper T-cell subsets into the tumors.

In a study by Gopalakrishnan et al. [31], patients with metastatic melanoma that responded to anti-PD1 therapy contained more diverse microbiotas than those that did not respond. Patients with a large abundance of one bacterial genus in particular, *Faecalibacterium*, were reported to be more than twice as likely to remain progression-free after six hundred days relative to patients with low *Faecalibacterium* abundance. Moreover, the abundance of *Bacteroidales* was positively correlated with the frequency of MDSCs and Treg cells. Another study by Matson et al. [32] reported enhanced T cell responses, and greater efficacy of anti-PD-L1 therapy, in germ-free mice reconstituted with fecal material from responding patients. This link between microbial composition and clinical response suggests a direct mechanistic influence on immunotherapy in human cancer patients.

Other specific examples of microbiota and their effects on ICI therapy include:

Akkermansia muciniphila: It has been reported that the response to PD-1 blockade from patients with renal cell carcinoma (RCC) and non-small cell lung cancer (NSCLC) was correlated with the abundance of *Akkermansia muciniphila*. Non-responders to PD-1 therapy were given oral supplements of *Akkermansia muciniphila*, which restored the efficacy of PD-1 blockade [30].

Bifidobacterium: In a preclinical murine model study, Sivan et al. (2015) [33] reported that oral administration of *Bifidobacterium* was effective in blocking tumor growth in melanoma-bearing mice. The enhanced antitumor response to *Bifidobacterium* treated mice compared to non-*Bifidobacterium* treated mice correlated with tumor-specific T cells in the periphery and accumulation of antigen-specific $CD8^+$ T cells within the tumor. Commensal *Bifidobacterium* can enhance antitumor immunity *in vivo* in an antigen-independent manner and also synergistically with PD-L1 blockade.

Although our knowledge of microbiota and its role in cancer is growing, there is still much that needs to be learned. The unearthing of the complex relationships between microbes and the TME will provide valuable insights into potential future cancer treatments [34].

Future strategies

Several mechanisms mediated by the gut microbiome can affect the therapeutics response and toxicity of ICIs, chemotherapy, and cell transplant [35]. It has been proposed that modulation of the TME microbiome may be a potential therapeutic strategy. The mounting evidence linking the gut microbiome to immunotherapy efficacy raises exciting opportunities to improve clinical outcome strategies. This is based upon the premise that antigen, adjuvant, and suitable immune microenvironment are responsible for the successful induction of anti-tumor adaptive immune response, all three of which are simultaneously impacted by the microbe environment [36]. Moreover, antibiotics can diminish the efficacy of ICIs, while conversely, specific gut microbes can enhance efficacy [37].

In addition to using the microbiome as a complementary prognostic or predictive biomarker of treatment outcomes, both preclinical and clinical evidence suggests that the relationship between the microbiome and patient responses is causal rather than correlative [38]. This allows for interventional strategies that involve manipulating the gut microbiome. The fecal microbiota transplantation (FMT) approach has been described for treating refractory *Clostridium difficile* infection in patients [39]. Other, more subtle, but less precise, approaches include modulating the commensal community through prebiotics or dietary changes to either favor the expansion of beneficial bacteria, or conversely, deprive detrimental bacteria of their required nutrients [40].

The response to both anti-PD-1 and anti-CTLA-4 immunotherapy can potentially be enhanced by modulating the gastrointestinal (GI) bacteria using oncomicobiotics [41]. Although the effect of both diet and probiotic supplements on this interaction is not well understood, one recent study on a cohort of 128 melanoma patients on ICI showed that sufficient dietary fiber, and no probiotic use, correlated with significant improvement in progression-free survival [42]. Moreover, in preclinical murine models that received a low-fiber diet or probiotics, there was an impaired response to anti-PD-1 therapy and a lower frequency of cytotoxic T cells in the TME. These results indicate that these factors ought to be incorporated into future strategies in patients receiving ICI therapy.

Given the intricacy of the commensal-host interaction, inter-individual variability, and microbiome diversity, it is likely that several modalities will contribute to the influence of the microbiota on immunotherapy efficacy. Future strategies of precision medicine may utilize companion diagnostics, in conjunction with therapeutic tools, to both identify and modulate the microbiome that diminishes therapeutic efficacy. Future strategies will likely integrate the relative contribution of the microbiome with other factors that affect the potency of immunotherapy [43].

Conclusion

The microbiome has emerged as a crucial factor influencing the balance between human health and disease. There is a growing understanding of the complex symbiotic relationship underpinning the biological mechanisms that link specific bacterial strains with host immunity. Since cancer immunotherapy has shown to be a promising treatment modality, evidence now indicates that the microbiome affects its therapeutic efficacy, particularly with respect to ICIs. Moreover, the therapeutic response or treatment-limiting toxicity of cancer immunotherapy can be influenced by gut microbiome modulation. Therefore, the integration of the microbiome with other factors that regulate the responsiveness of immunotherapy will facilitate enhanced

therapeutic outcomes. Further studies of the microbiome and intratumor microbiota are needed to explore their role in cancer and to discover new therapeutic strategies for cancer treatment.

Abbreviations

CD8: cluster of differentiation 8

CRC: colorectal cancer

CTLA-4: cytotoxic T lymphocyte protein 4

ICI: immune checkpoint inhibitor

IL-1: interleukin-1

PD-1: programmed cell death protein-1

TME: tumor microenvironment

Declarations

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Author contributions

The author contributed solely to the work.

Conflicts of interest

The author declares that there are no conflicts of interest.

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