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Targeting Gut Microbiota in Cancer Therapy

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Abstract. Our knowledge of the complex interactions between the gut microbiome and the numerous mechanisms by which they affect health and illness has greatly increased. Recent evidence suggests the composition of the gut microbiota can alter the effectiveness of both cancer radiation and immunotherapies. However, existing microbiota-based modification techniques including fecal microbiota transplantation, antibiotics, prebiotics, probiotics, and postbiotics are insufficient due to the wide variety and fluidity of the microbiota. With the advancement of next-generation sequencing and CRISPR techniques, future research will be able to accurately identify favorable and non-favorable microbial targets that can improve immunotherapy efficacy and expand the use of microbiota precision medicine.

Keywords: gut microbiota, cancer immunotherapy, composition

1. Introduction

It is generally understood that the hundred trillion microbes in the human gastrointestinal tract play a significant part in protecting human health [1]. They support basic bodily processes including nutrient extraction from food and drug metabolism. In terms of immunity, healthy gut microbiota can drive pathogens away by competing for resources and/or generating inhibitory substances. An imbalance (dysbiosis) in gut microbiota, on the other hand, may cause host machinery to malfunction, leading to the pathogenesis of a wide range of disorders, including cancer [1]. Accumulating evidence has shown that not only does microbial diversity affect cancer progression but also patients' responses to different cancer treatments [2]. However, the types of gut microbiota are highly unique between individuals and are affected by several endogenous and exogenous factors. The complexity of the composition poses a huge challenge in translating research findings into general cancer therapies. Latest advancements in sequencing and computational biology as well as CRISPR technology lay the path for future approaches that can modify these microbes more specifically to improve cancer treatment success and develop effective microbial targeting for personalized cancer treatment.

In this review, we will first give a general background on the gut microbiota composition, followed by its impact on cancer radiation- and immunotherapy. Then, we will highlight the success and limitations of current microbiota-based therapeutic strategies. Finally, we will discuss emerging techniques that advance the area and the path ahead.

2. Gut microbiota composition

The gut microbiota is a collective population of bacteria, archaea, viruses, fungi, and protozoans, with the two most common phyla, Firmicutes and Bacteroidetes, accounting for 90% of the total [3]. Despite phylum-level homogeneity, species-level microbial distribution differs greatly from person to

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person. The gut microbiota of each individual evolves as they age. Genetics, delivery technique, milk feeding practices, weaning duration, environmental exposure, and antibiotic usage all shape it starting from birth [4].

It is tricky to classify and group individuals together due to the variety and fluidity of gut microbiota. Enterotypes are a long-established approach, defined as clusters of bacteria that often coexist and dominate in one gut microbiota (2). Humans have been categorized into three enterotypes, Bacteroides, Prevotella, and Ruminococcus, which are mostly associated with long-term dietary habits. The Bacteroides enterotype was strongly linked with heavy consumption of protein and animal fat, such as the Western diet, whereas the Prevotella was associated with a high intake of sugar and carbohydrates [5]. There is no ideal gut microbiota composition, but a diverse and balanced host-microorganism is necessary to carry out metabolic and immunological processes correctly and prevent the onset of disorders.

3. Effect of gut microbiota Composition on cancer therapy

The gut microbiota exerts anti-tumor immunity through the activation of dendritic cells and cytotoxic T cells as well as short-chain fatty acids production, which can modulate the secretion of cytokines [6]. Researchers proposed that either the cross-reaction of gut microbiota antigens or the interaction between gut microbiomes and microbiome metabolites in the tumor microenvironment (TME) contribute to the enhanced anti-tumor response [7]. Interestingly, numerous studies have demonstrated a substantial correlation between better response rates and the richness and variety of cancer patients' gut microbiomes both in radiation- and immunotherapy.

A group led by Sims et al. is interested in the association between gut microbiome diversity and survival in cervical cancer patients receiving chemoradiation [8]. They discovered that a positive response to chemoradiation is related to the richness of the gut microbiota. Specifically, Porphyromonas, Porphyromonadaceae, and Dialister were highly represented in the feces of short-term survivors, whereas Escherichia coli, Enterobacteriaceae, and Enterobacteriales were dominating in long-term survivors' samples [8]. Furthermore, patients with a diverse microbiota had an increased level of CD4+ T cells that can detect malignancies, suggesting that altering the gut microbiota before chemoradiation may increase treatment effectiveness and enhance patient survival in patients with cervical cancer [8].

Jin et al. [9] observed similar findings when studying the influence of the gut microbiota on Chinese patients with non-small cell lung cancer who had undergone anti-programmed death 1 (PD-1) blockade. By comparing the gut microbiome profile of patients before and after the treatment, researchers discovered a positive correlation between a diverse composition and better memory T cell and natural killer cell activity as well as prolonged progression-free survival [9]. A composition analysis study also revealed these bacteria were considerably enriched in the responding patients [9].

4. Targeting gut microbiota in cancer: current strategies

Given the growing significance of the microbiome to anticancer immunity discovered by researchers, cutting-edge cancer treatment methods that alter the microbiota in the gut or even in tumor-associated tissues will become a key component. The use of fecal microbiota transplantation (FMT), antibiotics, prebiotics, probiotics, and postbiotics are just a few of the techniques that are currently being applied to target gut microbes. Numerous clinical studies examining these techniques in cancer patients are now ongoing or have already been completed. The effectiveness and limitations of these treatments are discussed here.

4.1. Fecal microbiota transplantation

FMT was first used to treat infections caused by Clostridium difficile [10]. By transplanting feces from a healthy person, FMT treatment boosts patients' gut microbial variety. FMT treatments may be given orally in the form of lyophilized tablets or via gastroscopy or colonoscopy [2]. Recent research

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suggests that FMT may be able to mitigate immunotherapy resistance to immune checkpoint blockade while also enhancing anticancer effects [2].

A phase I clinical study (NCT03353402) was carried out to assess the efficacy of FMT with reinduction anti-PD-1 therapy in 10 melanoma patients who had previously failed to respond [11]. Three of the 10 patients had tumor volume reduction, including two partial responses (PR) and one complete response (CR), according to the data [11]. Around the same time, a similar clinical trial was conducted to evaluate the safety of combining FMT with anti-PD-1 therapy in patients with melanoma (NCT03772899). In addition to providing evidence that the combination therapy is safe, the results supported that FMT enhanced anti-tumor responses in patients [12]. Elevated production of IL-17 was observed in post-FMT responders, which suggests an upregulation of T cell activation. The total response rate was 65%, with 3 CR [12].

Despite the positive outcomes of FMT in patients receiving immune checkpoint blockade, questions remain regarding its long-term safety. Numerous factors, especially choosing an appropriate donor, can severely affect how FMT responds. The FMT donor should ideally include a diverse range of microbial compositions, including bacteria that are advantageous for the particular type of cancer and immunotherapy. Bifidobacteria spp., Akkermansia muciniphilia, E. hirae, and Bacteroides spp. had previously been considered helpful bacteria that may boost anti-cancer response and better regulate tumor development in vivo [13]. Another potential issue is the actual transfer. To reduce the chance of transmitting infections to gut microbiota profile, meticulous and routine donor screening is necessary.

4.2. Antibiotic, prebiotic, probiotic, and postbiotics

While antibiotics are used to inhibit the growth of some undesirable gut microbiota, prebiotic, probiotic, and postbiotic supplements are often used to activate the favorable gut microbiome. Antibiotics are a two-edged sword. An advantageous example would be the use of antibiotics to suppress the prevalence of Fusobacterium nucleatum, which causes a poor prognosis for colorectal cancer (CRC) patients because it likely shields tumors from immune cell attack [14]. In a study led by Bullman, tumor sizes in the xenograft mice model made from the patient with CRC were successfully decreased by the antibiotic metronidazole, which F. nucleatum is sensitive to [15]. However, due to the lack of specificity, using antibiotics to modify gut microbiota might result in decreased microbial diversity, altered metabolic activity, or even an increase in pathogens that are resistant to antibiotics [13].

Prebiotics are dietary components that promote the growth of certain bacteria, which have the potential to enhance anti-tumor immunity [14]. For instance, Liu et al. [16] recently published a study evaluating the efficacy of a prebiotic bilberry anthocyanin combo on murine colon cancer patients treated with anti-PD-L1. They discovered that the combo improved intratumoral CD8+ T cell infiltrations and enriched certain subdominant species within the gut microbiota.

Likewise, probiotics are living microorganisms that improve the health of the host. They naturally produce postbiotics, which are functional bioactive substances or bacterial byproducts that can also improve health [14]. Probiotic effects in the clinic, however, continue to be dismal. Recent research reveals that using commercially available probiotic supplements is linked to inferior outcomes in immune checkpoint blockade-treated melanoma patients and preclinical mouse models [17]. Patients who did consume probiotics had considerably shorter progression-free survival. Similar results were observed in mice receiving probiotics, including a reduced infiltration ability of IF-γ-positive cytotoxic T cells in TMEs and a compromised response to anti-PD-1-based therapy [17]. Unlike antibiotics, there is very little research focusing on the beneficial and/or adverse effects of prebiotic, probiotic, and postbiotic supplements against cancer. Although numerous clinical trials are underway (NCT05122546, NCT03829111, NCT04857697, NCT03358511, NCT04362826), further investigations are necessary to validate and better understand the mechanism behind these techniques' therapeutic potential [14].

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5. Technology advancement & emerging strategies

5.1. NGS

Shotgun metagenomics and 16S rRNA gene sequencing (gene amplicon) are the two primary ways to identify the microbial species present in the gut [18]. The reasoning behind the 16S rRNA method is that the DNA sequence of the sections between the conserved regions of the 16S rRNA varies across bacterial species and may be utilized to distinguish closely related bacterial species. Hence, the amplicon-based sequencing uses PCR and compares the DNA sequence amplified to sequences of known bacterial species [19]. The shotgun sequencing method, on the other hand, uses a genome-wide strategy and splices the entire genomic DNA into short sequences and then aligns them to an annotated database of known DNA sequences. Shotgun sequencing is typically regarded as superior because it covers all the genetic material of a given sample, but it also leads to a higher false positive rate [20].

The advancement of next-generation sequencing (NGS) has fundamentally transformed 16S rRNA amplicon sequencing in terms of scale, speed, and cost-effectiveness. Several NGS platforms have been created, including the Illumina (MiSeq and HiSeq), Roche 454 GS FLX, Ion Torrent/IonProton/Ion Proton, SOLiD 5500 series, and Oxford Nanopore [18]. In a comparison of the Illumina MiSeq, Ion Torrent PGM, and 454 GS FLX Titanium platforms, Allali et al. [21] showed that despite few variations in diversity and abundance, all three platforms produce comparable biological findings, with GS FLX+ generating the longest reads and highest quality scores and MiSeq yielding the most reads after quality filtering.

As a result, the main difficulty with NGS data is translating the millions of sequencing reads to significant statistical and biological conclusions. Currently, the four most used bioinformatic pipelines are QIIME, Mothur, EBI, and MG-RAST, and tools for downstream statistical analysis and data visualization tools include Calypso and MicrobiomeAnalyst [18]. These developments have and will considerably advance the field of microbiota-based therapy research and provide intriguing new directions like the ‘next-generation’ probiotics [22]. Better bioinformatic tools must also be developed quickly to prepare for the growing demand for metagenomic data analysis.

5.2. CRISPR

Neither the FMT nor the use of antibiotics or probiotic supplements ensured that the imported or depleted microbes would not cause collateral damage to the patient's existing bacterial communities. The development of CRISPR technology enables direct editing of the gut microbes' DNA, adding a previously inaccessible level of precision to microbiome therapies. In a pioneering study, Lam et al. were able to alter the composition of the entire bacterial communities residing in the digestive tracts of mice by removing portions of the genes from the Escherichia coli bacteria that reside there [23]. This work suggests a promising future for expanding genetic manipulation to a broader spectrum of bacteria found in the human microbiome. However, finding the ideal phage for gene delivery will undoubtedly take further effort.

6. Conclusion

In conclusion, the composition of gut microbiomes has an impact on patients' responses to cancer therapy. However, existing methods for manipulating the microbiota to modify the response to immunotherapy are encouraging but have several limitations. The impacts of gut microbiota composition on cancer treatment are complex. Understanding gut microbiota effects in various tumor microenvironments require the ability to precisely identify immunostimulant and immunosuppressive strains as well as recognize advantageous and disadvantageous microbiome traits that target immune cells or pathways. Improvements must also be made to clinical trials' effectiveness and safety. Diet, drugs, host immune system, genetics, geographical factors, tumor type, and stage are just a few examples of elements that need to be closely monitored since they have an impact on the gut microbiota and may cause discrepancies across trials. Notably, improvements in sequencing and gene

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editing will serve as a pillar in the creation of novel microbiota-based approaches, such as engineered small-molecule drugs that activate metabolites or substances generated from gut microbiota, phage-based therapies, and more personalized care.

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