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Implication of gut microbiome in immunotherapy for colorectal cancer.

Gut microbiome in immunotherapy for colorectal cancer.

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Abstract

Colorectal cancer (CRC) constitutes the third most frequent malignancy reported in the male population and the second most common in women in the last decades. Colon carcinogenesis is a complex, multifactorial event, resulting from genetic and epigenetic aberrations, the impact of environmental factors, as well as the disturbance of the gut microbial ecosystem. The relationship of the intestinal microbiome with carcinogenesis was relatively undervalued in the last decades, however, its remarkable effect on metabolic and immune functions of the hostis under the spotlight in the recent years. There is a strong relationship between gut microbiome dysbiosis, bowel pathogenicity, and responsiveness to anti-cancer treatment, including immunotherapy. Modifications of bacteriome consistency are closely associated with the response to immunotherapeutic agents, a condition that implies the necessity of microbiome manipulation for the optimal response of CRC patients to immunotherapeutic agents. In this paper, we review the current literature on how gut microbiota influences the response of CRC patients to immunotherapy.

Key Words: Colorectal cancer; Gut microbiome; Immunotherapy; Checkpoint inhibitors; Tumor microenvironment

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Core Tip: Colorectal cancer constitutes the third most frequent malignancy. CRC is a complex, multistep process. The impact of environmental factors, as well as the disturbance of the gut microbial ecosystem is associated with CRC development. There is a strong relationship between gut microbiome and resistance to immunotherapy.

INTRODUCTION

Literature search

PubMed was searched to identify studies on gut microbiome, immunotehrpay and colorectal cancer. The literature review was completed on February 28, 2022. The following search terms were applied: "Colorectal cancer", "Immunotherapy" or "Checkpoint inhibitors" "Tumor microenvironment" "Gut microbiome". The reference lists of all related articles were screened for other potentially relevant studies. Finally, the authors similarly reviewed the reference lists of eligible articles to identify further eligible articles, books, and other forms of publication. Publications written in any other language other than English were excluded. Publications of abstracts were also excluded.

Introduction

Colorectal cancer (CRC) constitutes the third most frequent malignancy reported in the male population and the second most common in women in the last decades, based on GLOBACAN epidemiological data[1]. Colon carcinogenesis is a complex, multifactorial event composed of genetic and epigenetic aberrations, the impact of environmental factors, as well as the disturbance of gut homeostasis, resulting from gut microbiota modifications[2].The microbiome constitutes a multiplex ecosystem of microorganisms located in the gastrointestinal tract of many species, including humans [3].

The relationship of the intestinal microbiome with disease development, including carcinogenesis, was relatively undervalued in the last decades. However, the interrelation of gut microbiota with the main functions of the host is in the spotlight, for the last few years[4].The digestive tract contains the largest amount of microbiota colonization among other anatomical regions, presenting 70% of the whole amount of human microbiota [5], including viral and bacterial microorganisms, as well as, archaea and fungi[6, 7]. The proximal parts of the tract, including the stomach and small intestine, present few microbiota species in comparison with the distal part, where the colon presents the highest amount of species (microorganisms) in the colonic substance

[7]. The six main phyla of the gut microbiome (90% of the population) include[8], Bacteroidetes, Actinobacteria, Firmicutes, as well as Proteobacteria, Verrucomicrobia, and Euryachaeota [9]. From all the genera, Bacteroides constitutes the most plentiful (30%) in gut bacteriome [10], implying its significant impact on the human functional system, while many genera arising from Firmicutes phylum compose a high amountof the intestinal substance, such as lactobacillus, Clostridium, Faecalibacterium, Eubacterium, and Ruminococcus[11].The application of metagenomics on fecal specimens has given the opportunity for microbiome quantification and analysis, as well as it constitutes a potent diagnostic tool [12].

The functional role of the Gut microbiome

Gut microbiota exhibits diverse functions in the human organism, being responsible for many metabolic processes and biosynthesis. Vitamin synthesis constitutes one of the key roles of gut microbiota, such as riboflavin, vitamin B1, biotin, vitamin K, as well as cobalamin [13]. They also have a crucial role for the non-digestible carbohydrate metabolism and transform them into fatty acids (short-chain), such as butyric acid, acetic acid, as well as propionic [14], produced by the main phyla of bacteriome, including Bacteriodetes and Firmicutes [15]. Alteration of the above metabolic process leads to modification of the fatty acids production and metabolic imbalance [16].Except for their involvement in vitamin and short fatty acids synthesis, they take part in bile acid production [17]. Neuromodulators are also produced by gut microbiota, with a significant implicationfor the gut-brain axis, including the peripheral and central nervous system, as well as the enteric nervous system (ENS) [18]. Many neurological and psychiatric disorders are closely connected with the gut microbiome, due to the fact that it also synthesizes many pro-inflammatory cytokines, as well as amyloids and liposaccharides, in case of deregulation of the axis or dysbiosis of the microbiota [18]. Based on metagenomics, genome disturbance and dysbiotic flora predispose to many malignancies [19], as well as non-neoplastic disorders, such as atopy, functional intestinal

disturbances, like irritable bowel syndrome (IBS), as well as inflammatory bowel disease (IBD), and metabolic syndrome[20, 21].

Focusing on the gastrointestinal tract, there is a strong relationship between gut microbiome dysbiosis and bowel pathogenicity. In the case of bowel, functional disorders, such as irritable bowel syndrome (IBS), there are many studies that illustrate an altered consistency of the bacteriome, with an increased or decreased quantity of many bacteria. It is observed an aberrant increase of *Ruminococcus, Firmicutes*, as well as *Clostridium spp*. and an abnormal decrease of *Ruminococcusalbus and callidus and Bacteroidesfragilis*and *bulgatus* [18]. A connection between the overproduction of short fatty acids (SCFAs), that deregulate the secretion of serotonin from the enteroendocrine cells (EECs), leading to increased bowel movements and increased fermentation, which cause the symptomatology of meteorism [22]. In the case of organic bowel diseases, such as inflammatory bowel disease (IBD), including Ulcerative colitis (UC) and Crohn's disease (CD), an altered microbiome is demonstrated in patients who suffer from one of the above types of IBD. The modification of the gut microbiome was closely related to dietary habits [23]. Patients with CD demonstrate an increased amount, especially of Neisseriacaeacorrodens, *E. coli*, as well as proteobacteria [24], while an enhanced amount of fungal species was also reported such as *Candida albicans*, Cyberlindnera *jadinii*, and *Saccharomyces cerevisiae* [25]. Although a decreased quantity of some bacterial taxa such as Firmicutes, *Faecalibacterium prausnitzii*, Bacteroidetes, as well as Roseburia was observed[26]. Dietary habits including a high amount of fruits and vegetables consumption lower the risk for CD development [27].

Intestinal epithelial cells (IECs), are closely interrelated with the immune system *via* the existence of goblet and Paneth cells, as well as their products. Goblet cells are located in intestinal mucosa and they have a crucial role in producing mucus, while Paneth cells are located in the crypts of Lieberkühn, secreting various immunomodulatory peptides, with antimicrobial qualities[28]. Moreover, bacterial metabolites also take place in immune responses, *via* the production of SCFAs, which are closely associated with innate immunity and antibody production[29].

Immunotherapy constitutes a significant therapeutic option, including immune checkpoint inhibitors, cancer vaccines, as well as CAR-T cells [30]. This treatment modality makes use of the immune responses in order to create an anti-neoplastic effect. The main therapeutic agents include the following monoclonal antibodies: (i) anti-cytotoxic TT-lymphocyte antigen-4 (anti -CTLA-4), (ii) anti-programmed cell death 1 Ligand 1 (anti-PD-L1), as well as (iii) anti-programmed cell death protein 1(anti-PD-1[28, 31]. The principal advantage of immunotherapeutic agents includes their aimed action on malignant cells appears in **Figure 1**.

This therapeutic modality is currently selected as an anti-cancer treatment only in cases of tumors that are characterized by high microsatellite instability (MSI-H)[32]. Tumors that present MSI-H, arise either from a defective DNA mismatch repair (MMR) mechanism, that leads to the accumulation of genetic mutations, such as in the case of mutant MSH2, PMS2, MSH6, as well as MLH 1 or by epigenetic aberration, such as in case of genome hyper-methylation[33]. There are many reports that gut microbiota influences the response to anti-cancer treatment, including immunotherapy [34]. It is observed that many CRC patients that had a lack of specific taxa in their bacteriome, presented a limited response to immunotherapy agents such as anti-PD1 (anti-programmed cell death protein 1), a condition that implies their potent use for more successful personalized anti-cancer treatments. In this paper, we review the current literature on how gut microbiota influences the response of CRC patients to immunotherapy[35].

The role of Microbiome in colon carcinogenesis

There are many studies about the implication of gut microbiota in immunotherapeutic agents, including immune checkpoint inhibitors for melanoma. Fewer studies exist about its role in CRC treatment management.

Modifications in the gut microbiome and microbial metabolites have been involved in many pathological processes and diseases, including colon carcinogenesis[36]. Many intestinal bacterial products have been implicated in malignant states in the intestinal

tract [37]. Several studies demonstrate the presence of an altered microbiome either in CRC patients' fecal specimens or in malignant tissues compared to healthy ones[38]. These alterations in the microbiome take place in the initial steps of CRC development, which can be utilized as predictive biomarkers for patients with increased risk for colon adenomas that lead to CRC, as well as microbial diagnostic gene markers CRC [39].

Environmental factors have a high influence on the gut microbiome, as well as idiosyncratic factors[40], which subsequently induce carcinogenesis and CRC development, *via* the overgrowth of particular microbial species in the flora[41]. The formulation of colonic microbial substances is closely related to modifiable factors such as eating behavior and style of living [42], while it has a key role in the metabolism of nutrients[43]. There is a diversity of environmental risk factors that are associated with colorectal carcinogenesis such as obesity, tobacco use, alcohol consumption, and prepared meat products[44].

Many studies demonstrate the implication of specific bacterial taxa in carcinogenesis such as *Enterococcus faecalis, Helicobacter hepaticus*, as well as *Bacteroides fragilis*, and *Fusobacterium nucleatum*. The products of the above microbes lead to genomic alterations [45], while in the case of the latter microbe, the carcinogenesis indirectly occurs, *via* the perpetual secretion of pro-inflammatory cytokines [46]. The above phenomenon implies the close interrelation of the microbiome with immune response and metabolic processes[47].

There is a notable reduction of genera of Firmicutes phylum, which produce a significant metabolite, the so-called butyrate. An enhanced reproduction of specific phyla is reported, such as in the case of *Bacteroides fragilis, Peptostreptococcus stomatis* as well as *Tarvimonas micra, Fusobacterium nucleatum*[48], and *Solobacterium moorei*[49]. There are also reports that showed an increased amount of Enterococcus, Escherichia coli, as well as Klebsiella and Streptococcus and decreased Rothia [2].

There is considerable evidence that CRC development is closely associated with the presence of Fusobacteriaceae family members, such as *Fusobacterium nucleatum*,

necrophorum, as well as *Fusobacterium mortiferum* [37], *via* a mechanism, which was reported in mice[50].

In general, dysbiosis, including the modification of microbial taxa in the gut ecosystem, leads either to a limited variety of microbiota or the overgrowth of microbes, which can further lead to opportunistic infections[51], destruction of the intestinal epithelial barrier, bacterial translocation to the mesenteric lymph nodes (mLNs)or the circulatory system leading to the local and systemic inflammatory response[52].

Recruitment of T lymphocytes is observed in CRC malignant tissues [53], *via* the secretion of chemotactic cytokines, which is further related to abundance in proteobacteria, Ruminococcaceae, *B.fragilis*, as well as *E.coli*. On the contrary, a high amount of Fusobacteriais associated with a dismal prognosis, expressing *in* vitro an increased amount of recruited T cells and inflammatory modulators (Il-6, IL-8, IL-1)[54], an inhibitory effect on Natural killer cells (NK cells), as well as in the tumor-infiltrating lymphocytes [55]. Although Fusobacterium nucleatum is relatively associated with a worse prognosis, it constitutes a promoter of differentiation for Regulatory T cells (Tregs) with decreased expression of scurfin or FOXP3 (forkhead box P3), which is correlated with prolonged survival[56].

Immunotherapy in CRC

The therapeutic management of CRC is considered quite challenging, due to the multiplex molecular basis, including genetic and epigenetic alterations, under the influence of external factors[57]. In recent years, immunotherapeutic agents are utilized for tumors that present high microsatellite instability (MSI-H), resulting from a defective DNA mechanism(MMR)or epigenetic modifications[33]. An epigenetic aberration is genome hyper-methylation, while there are reported mutant genes such as PMS2, MLH1, as well as MSH2, and MSH6[58]. In the case of MSI-H colorectal tumors, methylation of CpG islands in the promoter of BRAF proto-oncogene [59], while it is observed that patients with BRAF and RAS genetic mutations present resistance to immunotherapeutic treatments with a limited enhancement of survival [60], such as in

cases of epidermal growth factor receptor inhibitors (EGFRIs), like cetuximab, as well as Panitumumab[61]. In comparison with MSI tumors, the microsatellite stable tumors present a more aggressive phenotype and worrisome prognosis[62]. Immunotherapeutic agents, such as Pembrolizumab are commonly used in cases of chemo-resistant advanced colorectal malignant tumors, despite the existence or lack either of MMR or MSI-H, based on KEYNOTE 028 clinical trial[63], while for tumors with MMR phenotype, the utilization of nivolumab alone or with ipilimumab is highly recommended[47].The administration of cancer vaccines in CRC is still under study and it is limited only to cases of end-stage CRC[64]. Talimogene laherparepvec vaccine uses as a vector Herpes virus type-1 and aims at the GM-CSF gene. The combination of Systemic use of atezolizumab (anti-PD-L1 immunotherapeutic agent) with the above vaccine is currently under assessment for tumors with microsatellite stability[63] or as monotherapy in secondary liver cancer[65].

4.1 Tumor microenvironment and microbiome in CRC

Tumor microenvironment (TME) includes multiple types of cells, such as fibroblasts immune cells, as well as endothelial and stromal cells[66]. TME has a quite significant role in immunes responses, particularly in CRC, while it constitutes a therapeutic target for many anti-cancer agents[67]. The stroma around the tumor has a key role in unresponsiveness to chemotherapy, due to the fact that it includes a heterogeneous population of cells with various levels of differentiation, that contribute to tumor invasive behavior and dissemination, such as in the case of Tumor-associated macrophages (TAMs) and Cancer-Associated Fibroblasts (CAFs), which are related with a dismal prognosis and neoangiogenesis[68, 69], as well as Myeloid-derived suppressor cells (MDSCs), which are also implicated in tumor progression and invasion, while their effect is under the regulation of tumoral products, like chemokine (C-C motif) ligand 2 and 5 (CCL2 and CCL5)[70].

As it was underlined, that gut microbiota exhibit various effects on differentiation mechanism and tumor development, while they influence the tumor response to immunotherapeutics [71]. The existence of intra-tumoral bacteria is reported in many solid tumors, especially in breast cancer, while it was demonstrated, that microbiome is particular for each kind of malignant tumor, presenting distinct metabolic functions [72]. Based on data that are collected by whole-transcriptome analysis there is a distinct microbiome, correlated with different malignant tumors, implying a specific microbial profile for each type of cancer[73]. TME also has a crucial role in the existence and multiplication of intra-tumoral bacteria[74].Many studies illustrate the close relationship between immunotherapy and gut microbiota and their implication for the anti-tumor response, such as immune-checkpoint inhibitors [72].

The implication of gut microbiome in immunotherapy

Resistance to immunotherapy is difficult to be overcome in clinical practice[31]. Manipulation of gut microbiota constitutes a promising way of reducing the resistance to therapeutic agents, which is implied by the notable effect of intestinal microbial products on the malignant tumor, which could also be considered cancer-driving molecules[75].

Experimental studies on mice have shown that bacteria have a crucial role in the anti-cancer immune response, while the response was limited in the case of germ-free mice [28]. It was primarily reported in mice that intestinal microbiota have a significant role in the response especially to immune checkpoint inhibitors, however, the above knowledge was also demonstrated in humans that immune checkpoint blockade was applied[28].In mouse-model studies, fecal microbial transplantation (FMT), from mice with immune-responsive microbiota to germ-free, provided a better anti-neoplastic response and tumor growth management for the latter, a result which was associated with an increased amount of cytotoxic T lymphocytes (CD8+) in TME[76], whereas transferring of fecal-samples including microbiota prone for carcinogenesis, provided

the opposite results to physiological mice [77]. However, the correlation of the anti-tumor response with external influencing factors must be taken into consideration.

Alterations in the consistency of bacteriome were reported in cases of patients with an active response to PD-1 inhibitors. More specifically, these patients presented a higher amount of *Enterococcus faecium*, *Bifidobacterium longum*, as well as *Collinsella aerofaciens*. Fecal specimens that presented the above microbial taxa, were characterized as "responder" stool samples and they were transferred *via* FMT to germ-free mice. Then, the germ-free mice started to express the stool phenotype of the responders[28].

Based on various human or animal-model cohort studies, intestinal microbiota, could not only have beneficial but also toxic effects on the immune checkpoint inhibition [78]. Reduced toxicity was observed in specimens where Bacteroidetes genera were in abundance, although they are connected with unresponsiveness to ICIs, in contrast to Firmicutes, especially in the case of Ruminococaceae, which were not only responsive to ICIs but also presented toxic effects, whereas, in cases of overgrown *Faecalibacterium prausnitzii*, patients had an increased risk of presenting colitis related with CTLA-4 inhibitors[79, 80].

5.1 Manipulation of intestinal microbiota for immunotherapy- response improvement

Based on all the aforementioned characteristics of the intestinal microbiota either can promote the anti-neoplastic response or can induce inflammation and carcinogenesis[81]. A reduced host anti-cancer response was observed in Germ-free mice or in the case of antibiotics administration (broad-spectrum) [28, 35]. In the case of urinary tract malignancies and lung cancer, the antibiotics had a harmful effect on anti-PD1/PD-L1 treatment[35], in comparison with cyclophosphamide which presented a promoting effect for the overgrowth of *Barnesiella intestinihominis* in the intestinal tract and a stimulatory effect on anti-cancer immune response[82].

Manipulation of microbiota and the utilization of antibiotics for the killing of bacteria that are detrimental to the response to immunotherapeutic agents. However, the use of

antibiotics reserves the risk of killing also favorable bacterial species. In order to avoid the non-selective effect of antibiotics, bacteriophage therapy is applied, which permits a selective elimination of unfavorable bacteria [83].

Last but not least, environmental and lifestyle habits, could potentially alter the gut microbiome, such as physical exercise, proper dietary habits, and sleep patterns, as well as the utilization ofFMT[84].Bacteriotherapy or FMT, includes the transferring of beneficial bacterial species such as Bacteroides, Bifidobacteria, as well *E. hirae* and *Akkermansia muciniphilia*[85].

CONCLUSION

The relationship of the intestinal microbiome with disease development and carcinogenesis was underestimated in the last decades. Nevertheless, the crucial role of intestinal microbiota is in the spotlight the recent years, not only for their significant influence on the main metabolic functions of the host, but also on the immune and anti-tumor responses. Immunotherapeutic agents are commonly used especially in cases of chemo-resistant advanced colorectal malignant tumors. The implication of gut microbiota in the anti-cancer immune response is still under research, however, there are many reports that support that the lack of specific bacterial taxa in CRC patients leads to a limited response to immunotherapy or to complete unresponsiveness, while the presence of specific phyla could promote the anti-cancer response. Based on various human or animal-model cohort studies, intestinal microbiota, could not only have beneficial effects on immune checkpoint inhibition, but also detrimental effects. The aforementioned phenomenon illustrates the necessity for the manipulation of intestinal microbiota, for the highest anti-neoplastic immune response, either *via* bacteriophage therapy, or lifestyle habits modifications, as well as FMT . Although, further research about the implication of gut microbiome in immunotherapy response is needed, for the identification of additional druggable targets and the manipulation of intestinal microbiota for the achievement of an optimal therapeutic response, personalized for each patient.

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