Journal of Mind and Medical Sciences

Volume 8 | Issue 2 Article 8

2021

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Journal of Mind and Medical Sciences

https://scholar.valpo.edu/jmms/
https://proscholar.org/jmms/

ISSN: 2392-7674

Intestinal dysbiosis – a new treatment target in the prevention of colorectal cancer

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ABSTRACT

The gastrointestinal microbiome contains at least 100 trillion microorganisms (bacteria, viruses, fungi), whose distribution varies from the mouth to the rectum spatially and temporally throughout one's lifetime. The microbiome benefits from advancing research due to its major role in human health. Studies indicate that its functions are immunity, metabolic processes and mucosal barrier. The disturbances of these functions, dysbiosis, influence physiology, lead to diabetes, inflammatory bowel disease, obesity and colon tumorigenesis. The third most common form of cancer, colorectal cancer, is the result of many factors and genes, and although the link between dysbiosis and this type of cancer is poorly characterized, it has been shown that some bacterial species and their metabolites have a critical role in developing colorectal cancer. Also, gut microbiota plays a role in the inflammatory response and immune process perturbations during the progression of colorectal cancer. Some new technologies, such as metagenome sequencing, facilitated the progress by analyzing the metabolic and genetic profile of microbiota, revealing details about the bacterial composition, host interactions, and taxonomic alterations. This review summarizes the studies regarding the link between gut microbiota and colorectal cancer, targeting new therapeutic strategies.

Category: Review

Received: June 8, 2021
Accepted: August 17, 2021
Published: October 10, 2021

Keywords:

intestinal dysbiosis, colorectal cancer, therapy, prevention

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Introduction

Dysbiosis or the alteration of the gut microbiome's composition has recently been associated with various human diseases, including even cancer [1]. A possible mechanism through which the microbiota could influence carcinogenesis is the biosynthesis of chemical carcinogens such as N-nitroso compounds or acetaldehyde by microbes, associated with dysbiosis-related inflammation [2]. It was estimated that more than 70% of the human microbiome is located in the colon, so this part of the digestive tract is considered to be its most heavily colonized section [3]. It may be the reason why the colon is more prone to developing cancer, in contrast with the small intestine (the cancer incidence is 12-fold higher in the colon compared to the small intestine) [4]. It is important to know that the lifestyle and the dietary habits corroborated with other risk factors including advanced age, impaired glucose metabolism, pro-inflammatory states, previous therapies such as antibiotic therapy, chemotherapy, co-morbidities modulate the gut microbiota; that is maybe why the microenvironment created by the alterations of the typical resident colonic flora could be more favorable to tumor development at this level [5-8]. Many studies report specific alterations in the gut microbiome associated with colorectal cancer; that is why it was taken into consideration as a screening method, being very important in the early diagnosis (early diagnosed patients with colorectal cancer (stages 0, I or II), have 80% survival rates over five years in contrast with the ones diagnosed in stage

To cite this article: Florinela-Andrada Dumitru, Sergiu Ioan Micu, Roxana Emanuela Popoiag, Marilena Musat, Andreea Daniela Caloian, Valentin Calu, Vlad Denis Constantin, Daniela Gabriela Balan, Cornelia Nitipir, Florin Enache. Intestinal dysbiosis – a new treatment target in the prevention of colorectal cancer. *J Mind Med Sci*. 2021; 8(2): 221-228. DOI: 10.22543/7674.82.P221228

IV that have only a 10% survival rate). Therefore, a promising strategy for the early diagnosis of colorectal cancer is the detection of specific microbiome alterations [9-13].

Discussions

1. Human microbiota

1.1 Microbial species

The human body is colonized by a complex of the symbiotic, commensal and pathogenic microbial community. The human microbiome does not only include bacteria, but also fungi, protozoa and viruses [14,15]. A comprehensive group of living organisms considered a domain of life themselves are bacteria, many major bacterial phyla compose the human microbiota. Among them, the majority can be assigned to four types: Firmicutes, Bacteroidetes, Actinobacteria and Proteobacteria [16]. Firmicutes and Bacteroidetes represent more than 90% of the gut microbiome [17]. Firmicutes are composed mainly of Clostridia (Gram-positive and anaerobic) and Bacilli (obligate or facultative aerobics) [18,19]. Actinobacteria (for example, Bifidobacterium) are multiple branching rods, Gram-positive, non-spore-forming, non-motile and anaerobic bacteria. The phylum Bacteroidetes is composed of non-spore, Gram-negative bacteria. Proteobacteria (for instance, Escherichia, Klebsiella, Enterobacter) are aerobic or facultative anaerobic, non-spore-forming rod, Gram-negative bacteria [20].

There is a concept called “enterotype clusters”, through which the microbiota structure and different microbial colonization are better defined in various cohorts. It also allows the classification of each person by the relative abundance of specific bacterial in the fecal samples. The results of the studies performed on Japanese, American and European subjects confirm three clusters dominated by Bacteroides (enterotype 1), Prevotella (enterotype 2) and Ruminococcus (enterotype 3); each one of these three types is characterized by the relative abundance of metabolic pathways and by specific taxonomic composition [21].

1.2 Inflammatory and immune responses

The microbiota composition varies according to the various locations along the gastrointestinal tract. Microbial dysbiosis can be the result of the dysregulation of the intestinal immune system.

There is a delicate balance between the immune system and immune-regulatory functions, because the loss of a specific species can suppress the innate immune system or an overreaction. There is an immunological and physical barrier in the intestinal mucosa, between the luminal bacteria and the underlying immune cells, called intestinal epithelial cells; hematopoietic cells and intestinal epithelial cells generate various receptors named pattern recognition receptors; these mediate the interactions between the commensal microbiota and the immune system [22]. Toll-like receptors and nuclear oligomerization domain-like represent some pattern recognition receptors; these recognize microbial molecules (microbe-associated molecular patterns - lipid A, lipopolysaccharides, flagella, peptidoglycans and microbial RNA/DNA) and activate inflammasomes such as TNF alpha, cytokines and IL-1 beta [23].

It is essential to understand how and when dysbiosis and the genetic defects in the mucosa-intestinal epithelial cells and innate regulatory mechanisms can lead to the development of inflammatory or infectious diseases [24].

1.3 The metabolic role

Recent studies have revealed that the gut microbiota can generate genotoxic or metabolite stress in the intestinal environment and thus, genetic and epigenetic changes are facilitated and lead to cancer. As to provide the repair of DNA damage and energy for multidrug resistance efflux pumps, glycolysis will generate adenosine triphosphate and nicotinamide adenine dinucleotide for the polymerase. That is why enhanced glycolysis can have an impact on drug resistance. Glucose metabolism can be improved by dietary fiber that increases the abundance of Prevotella. An increased amount of Prevotella in the gut microbiota protects against Bacteroides induced glucose intolerance. The last one may facilitate drug resistance and also could affect the tumor growth. Increased glycolysis enables the diversion of glycolytic metabolites into other important biosynthetic pathways that play an essential role in cell proliferation. That is why the control of gut microbiota and their metabolites can be a useful strategy in reducing drug resistance [25,26].

It has been shown that chronic low-grade inflammation, promoted by lipopolysaccharides, is linked to the occurrence of insulin resistance and obesity. These endotoxins may cross the gut mucosa by infiltrating chylomicrons or weak intestinal junctions, stimulating the absorption of cholesterol and dietary triglycerides from the gut to the plasma, producing a natural immune response. Compared to healthy individuals, the level of circulating endotoxins was 20% higher in people with glucose intolerance or obesity, and 125% higher in type 2 diabetes individuals. These elevated levels were also correlated with high concentrations of TNF- α and IL-6 in adipocytes. Also, a diet poor in fruit and fiber, but rich in high-carbohydrates or high-fat, triggers the systemic secretion of lipopolysaccharides, the expression of Toll-like receptor 4, nuclear factor κB and the suppression of cytokines. These factors regulate the pathways involved in insulin secretion [27-30].

The microbiota plays an important role in many aspects of the metabolism and brain functions. Several studies reveal the importance of microbiota and micronutrients in

attention deficit hyperactivity disorder in children. Actinobacteria abundance may have a role in neuropsychiatric disorders, while Bifidobacterium spp are considered to have a protective role by several studies, while in other studies, they seem to be key-driver [31-33]. Regarding the micronutrients, increased Copper and Copper/zinc ratio are associated with attention deficit. Dysbiosis and the destruction of the intestinal barrier function was found to be a risk factor for the occurrence and worsening of the chronic kidney disease. The mechanisms involved are chronic inflammation and increased productions of uremic toxins [34-37].

1.4 DNA damage

The accumulation of DNA deteriorations destabilizes the human genome, promoting aberrant cells (pre - / cancerous) to preserve or to accelerate mutations. Gut microorganisms may be a serious source of DNA mutagens. For instance, studies have proven that some Enterococcus spp provide reactive oxygen species, such as hydroxyl free radicals, that cause DNA alterations, point mutations, protein-DNA crosslinking, destabilizing colorectal cancer-related genes. Enterotoxigenic Bacteroides fragilis triggers spermine oxidase expression on colonic epithelial cells, promoting DNA deteriorations. Furthermore, PKS-positive Escherichia Coli stimulates DNA double-strand breaks, cell cycle arrest and aneuploidy cell division. All these findings prove that microorganisms may induce DNA alterations and destabilize the genome directly or indirectly. Thus, the gut microbiome may be a risk factor and a treatment objective in colorectal cancer [38].

1.5 The modulation of cell proliferation

To control the cell function and structure in normal tissues, there are signals that control the cell's growth and death. When perturbations of apoptotic signals and cell proliferation develop, they will promote continued cell proliferation, thus forming malignant tumors. Enterotoxigenic Bacteroides fragilis drop Bacteroides fragilis toxin (fragilysin) to promote E-cadherin cleavage, promoting beta-catenin nuclear translocation, transcriptional upregulation of the proto-oncogene c-Myc, and colonic cell proliferation. Identically, fusobacterium nucleatum connects to E-cadherin through FadA, triggering downstream pathways and promoting cell proliferation. In conclusion, studies have proven that dysbiosis is a cause of aberrant epithelial cell proliferation and early tumor development [37].

2. Gut microbiota and colorectal cancer – is there any link?

Colorectal cancer is the fourth most frequent type of cancer worldwide, with a total of 8.5% of all new cases of cancer/ year, thus being a major health problem [39]. The development of this complex malignant disorder contributes to a large amount of factors, including environmental risk and genetic factors [40]. Considering that less than 20% of the cases are hereditary, we can conclude that environmental risk factors play a decisive role, including alcohol consumption, smoking, diabetes, obesity, a diet rich in animal fat, processed foods, red meat, low intake of fruits and fibers [31,40]. Studies show that genetic and epigenetic alterations in proto-oncogenes, tumor suppressor genes and DNA repair genes, contribute to the tumor transformation of the normal colonic epithelium. Recently, studies have focused on gut microbiota examination, and reported alterations in colorectal cancer samples, indicating that the microbiome may be a crucial factor in the induction and evolution of colorectal cancer [41-43].

The gut microbiota contributes to the epithelial barrier function and mucosal homeostasis, efficiently keeping bacteria into the lumen. These function perturbations promote an increased intestinal permeability, which is correlated with many gastrointestinal diseases, such as irritable bowel syndrome, celiac disease, inflammatory bowel disease, and colorectal development. The gut microbiota metabolic products may also influence the immune response, activating pro-inflammatory mediators, such as interleukin-6, cytokines, and tumor necrosis factor – α, leading to epithelial cell damage [44]. Furthermore, bacterial species can cause inflammatory responses or produce toxins, thus damaging the epithelial cells directly. Examples: species of Bacteroides fragilis and Enterococcus faecalis generate enterotoxins and reactive oxygen inducing oxidative DNA damage; Bacteroides fragilis induces cellular proliferation by activating β-catenin nuclear signaling; Fusobacterium nucleatum adheres and invades epithelial cells by the FadA surface protein [45,46].

Studies have shown that plenty of these bacterial species are found in different amounts in healthy individuals compared to colorectal cancer samples. Thus, microbiota in colorectal patients contains especially pro-inflammatory opportunistic microorganisms and pathogens associated with metabolic disorders, and a reduced amount of butyrate-producing bacteria, which has a significant role in intestinal homeostasis [47]. Moreover, some bacterial species have a high prevalence in colorectal patients than healthy populations: Escherichia coli, Bacteroides fragilis, Enterococcus faecalis, Streptococcus gallolyticus, Fusobacterium nucleatum. Instead, types of bacteria such as Roseburia, Clostridium, Faecalibacterium and Bifidobacterium are reduced [48]. Studies focused on discovering oncobacteria, but no single species have been universally detected in all colorectal patients. This indicates that various associations of microorganisms may

act synergistically, and the modifications in both damaging or protective bacterial populations may be responsible for the development and evolution of colorectal cancer [49].

3. Microbiome – a tool in the early diagnosis of colorectal cancer?

The development of colorectal cancer from normal mucosa is a complex multi-step process, from pre-neoplastic lesions to adenomatous polyps to carcinoma proliferation, which may take about ten years to occur. This is the main reason why colorectal cancer is a malignancy that may be screened. Many countries have initiated population screening and prevention programs, using fecal occult blood test and the fecal immunochemical test, followed by colonoscopy when the tests are positive. Thus, precancerous proliferation may be detected, with a highly successful treatment, preventing colorectal cancer and improving the overall survival [50,51].

Current studies have focused on additional criteria that may be considered in the algorithms used to lead patients to colonoscopy, including molecular biomarkers connected to the processes of carcinogenesis, such as circulating tumor cells and cell-free DNA microRNAs, and metabolites from plasma samples [52].

As discussed above, microbiome alterations are associated with colorectal cancer, so the option of using different microbiome stool signatures has emerged. Even though there is no major difference in the overall composition of microbiota in colorectal patients and healthy individuals, the distinction involves plenty of key microorganisms. This is one of the reasons why colorectal cancer diagnosis needs to develop some specific tests for those suggestive species, without evaluating the whole microbiome. For example, Fusobacterium nucleatum has been persistently discovered in large amounts in the feces of patients with adenoma and colorectal cancer, compared to healthy humans and cancer tissue compared to circumferential normal tissue [53].

Even though efforts have been conducted to discover the bacterial biomarkers that may predict the risk of colorectal cancer, major factors such as genetics, lifestyle, and environmental factors are known to influence the composition of gut microbiota. Also, the quality of samples, experimental protocols, and bioinformatics tools are all influencing factors and may explain the differences between studies [54]. Regardless of the advances in bacterial signatures in colorectal cancer patients, the clinical routine is still not using microbiome biomarkers in the screening of colorectal cancer.

In conclusion, the purpose of all this research is finding a superior prediction on colorectal cancer development, evolution and treatment strategies, by incorporating information on genetics with the microbiome, lifestyle, environmental factors to create new risk models.

4. Dysbiosis – a treatment target of colorectal cancer

The possibility of modulating the gut microbiome is a new therapeutic strategy in preventing and treating some disorders that are in direct relationship with the microbiota alterations. Considering the possible connection between colorectal cancer and microbiota previously mentioned, there is an increasing interest in discovering microbiome-related treatments. However, therapy strategies include the use of pre- and probiotics, fecal microbiota transplantation and phage therapy [55].

Fecal microbiota transplantation has been used as a therapeutic method in inflammatory bowel diseases, irritable bowel disease, metabolic syndrome, obesity, atopy, multiple sclerosis, and favorable outcomes. There are no conclusive data from clinical trials in treating colorectal cancer by using fecal microbiota transplant [56].

Another direct strategy is using antibiotics to modulate the microbiome and indirectly influence colorectal cancer evolution, thus targeting some bacteria (Fusobacterium nucleatum, Bacteroides fragilis, Escherichia coli). One study showed that the therapy reduced the amount of Fusobacterium nucleatum and the tumor's overall growth. Still, antibiotics may lead to dysbiosis and drug resistance. Therefore, there is a need for new products targeting bacteria more specifically. For example, a glycopolymer, antagonist of the Escherichia Coli virulence factor FimH, exposed a reduction in bacteria's adherence to the gut epithelia [57]. Furthermore, a low dose of a recombinant BFT-2 enterotoxin, which is a virulence factor of Bacteroides fragilis, reduced the tumor's development in the colorectal cancer mouse model [58]. Another major factor is the interaction between the microbiome and the viral component, and its modulation by phage therapy, due to its role in combating antibiotic-resistant microorganisms [59].

Another promising treatment strategy, affecting the immune response and metabolic pathways, is diet-keeping and vitamin D administration as an anti-neoplastic effect. [60] It is one of the most significant factors shaping the human microbiome, as studies have shown that alterations induced by deoxycholic acid developed carcinogenesis. At the same time, high dietary fiber and butyrate-producing bacteria may considerably reduce tumor growth [61]. Studies showed that the microbiome might affect the response of 2 cancer immunotherapeutic agents (anti-CTLA4 and anti-PD-L1), by amplifying the activation of dendritic and anti-tumor T cells responses [62-64]. Also, the immunologic stimulation by Bacteroides spp and Bifidobacterium spp affects the efficacy of the therapy. Furthermore, it has been proven that the imbalance of dietary sphingolipids may influence the effectiveness of chemotherapy and radiotherapy [64].

Probiotics regulate pathogenic microorganisms and the immune response, which may reduce blood cholesterol, colitis and prevent colorectal cancer, using different mechanisms: releasing detoxifying agents, anti-inflammatory factors, anti-carcinogenesis compounds (anti-angiogenesis), short-chain fatty acids that increase the intestinal barrier function [65]. For example, butyrate-producing species such as Clostridium butyricum and Bacillus subtilis may have an anti-tumor effect; Lactobacillus casei (strain BL23) may suppress colorectal cancer by targeting intestinal dysbiosis [66], while Lactobacillus casei (strain ATCC 334) produce ferrochrome which prevents colorectal cancer evolution by apoptosis mediated through the c-Jun N-terminal kinase pathway [67], and Lactobacillus casei (rhamnosus Lcr35) inhibit intestinal mucositis [68,69].

As already mentioned above, the intestinal microbiome, through the stimulation of the immune system, has a natural anti-carcinogenesis effect, so, it has been suggested as a key mechanism of cancer immunotherapy. There are three known ways: microbial antigens activate T-cell response with activating tumor-specific immune response (Bifidobacteria spp, Akkermansia muciniphilia, Bacteroides spp, Enterococcus hirae), the activation of pattern recognition receptors that mediate pro-immune and anti-inflammatory effects and the release of small metabolites that mediate systemic effects in the host (polyamines, vitamin, desaminotyrosine) [70].

All these data show the importance of new treatment strategies in different types of cancer, including colorectal cancer, meaning combined chemo- and immunotherapy with adjuvant therapies aiming the intestinal microbiota, such as diet, pre- and probiotics and vitamin D supplementation [71].

Conclusions

Despite the implemented screening programs many countries have already adopted, colorectal cancer remains a serious public health issue, being the third leading cause of cancer death. For this reason, research must continue to explore new strategies regarding the prevention, prediction, diagnosis and treatment options. Considering the major roles gut microbiota has on metabolism, nutrition and the immune system, as well as, tumorigenesis induced by DNA alterations due to dysbiosis, further research is expected to discover specific microorganisms and their metabolites connected to colorectal cancer induction and progression. This may provide new tools in the clinical management of colorectal cancer patients.

Conflict of interest disclosure

There are no known conflicts of interest in the publication of this article. The manuscript was read and approved by all authors.

Compliance with ethical standards

Any aspect of the work covered in this manuscript has been conducted with the ethical approval of all relevant bodies and that such approvals are acknowledged within the manuscript.

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