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INTESTINAL MICROBIOTA COMPOSITON AND THE RISK OF COLORECTAL CANCER - NOVEL APPROACH OF AN OLD ISSUE

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Abstract

Colorectal cancer is a multifactorial disease involving genetic, environmental and lifestyle risk factors. As a good example of chemical carcinogenesis, the recent studies increased a role of the intestinal microbiota in the development of this disease. This review is an attempt to summarize the current knowledge about the potential links between the intestinal microbiota, dietary style and colorectal cancer, emphasizing that changes in the intestinal microbiota composition, interfere with cell cycle regulation and the production of toxic metabolites that have deleterious effect on colorectal mucosa.

Different electronic databases such as PubMed, Google Scholar, and Web of Science were searched for relevant literature which has been reviewed in this article. We found that in this literature, several bacterial species have been shown to exhibit the pro-inflammatory and pro-carcinogenic properties, which could consequently have an impact on colorectal carcinogenesis. On the other side bacterial microbiota modifications through dietary style changes, could represent novel prognostic markers and/or targets for innovative therapeutic strategies. It means that exploitation of the gut microbiota offers opportunities for the personalization of chemotherapeutic regimens and the development of novel therapies for colorectal cancer patients.

Diversity of the microbial ecosystem favors organism homeostasis, particularly at the level of the cancer-immune dialogue. Therefore, the gut microbiota is both a target for nutritional intervention and a factor influencing the biological activity of other food compounds acquired orally, as well as a moderator of effective anticancer therapy.

***Key words**: Colorectal cancer, Microbiota, Anticancer therapy, Dietary style.*

1. Introduction

The human intestinal tract contains about 10^{14} bacteria, comprising about one thousand bacterial species, which are essential for fooddigestion. These are also responsible inintestinal epithelial homeostasis-control, intestinal development and human health, generally [1, 2]. Conversely, a large body of evidence supports a relationship between infective agents and human cancers and suggests that certain mucosa-associated bacterial species play an important role in the pathogenesis of colorectal cancer (CRC) [3, 4]. Recent publications have provided evidence for the involvement of gut bacteria in the development of CRC, which comprises, production of DNA damaging superoxide radicals, production of genotoxins, T helper cell-dependent induction of cell proliferation, and Toll-like receptor mediated induction of pro-carcinogenic pathways [3, 5, and 6]. Unfortunately, thus far, no clinical data have been available to show directly the distinct bacterial colonization patterns in CRC patients.

In fact, the molecular nature of the complex intestinal community was largely unexplored prior to the moment that Eckburg and coworkersrevealed the presence of ~400 bacterial species by sequencing prokaryotic ribosomal RNA gene sequences from multiple colonic mucosal sites and feces of healthy subjects [7]. Further investigations revealed high intra-individual variation of intestinal microbiomes in the human population, whereas the microbial colonization of the mucosa within adult individuals is relatively stable throughout the colon [8, 9]. Based on the latter observations we hypothesized that the in-depth analysis of a relatively small number of paired on/off-tumour tissue samples from CRC patients could disclose bacterial species that might be implicated in CRC etiology.

The data provided in this study is among the first "images" of the human CRC microbiome that highlight the fact that CRC is associated with quite dramatic shifts in the adherent intestinal microbiota.

2. Gut microbiota

Cancers are common chronic diseases worldwide and cause severe health burdens. There have been ongoing debates on the role of gut microbiota in the prevention and management of cancers, thus, it is worthwhile to pay high attention to the impacts of gut microbiota on several cancers, such as colon, and liver especially [3, 9, and 10]. In addition, it has been reported that gut microbiota may also affect the efficacy of cancer chemotherapy and immunotherapy. Among all the factors that influence gut microbiota, diet is the most influential and modifiable. The prebiotics, dietary fibers, short-chain fatty acids, and other bioactive compounds are all important dietary components to assist the growth of beneficial microbiota in the gut, which can protect against cancers and promote human health [9-11]. Their beneficial effects can be due to the fermentation of dietary fibers, the metabolism of phytochemicals, the synthesis of estrogens, and interactions with chemotherapies and immunotherapies [11].

According to Lucas *et. al.*, [4], several bacterial species have been shown to exhibit the pro-inflammatory and pro-carcinogenic properties, which could consequently have an impact on colorectal carcinogenesis. Some bacterial species have been identified and suspected to play a role in colorectal carcinogenesis, such as: *Streptococcus bovis, Helicobacter pylori, Bacteroides fragilis, Enterococcus faecalis, Clostridium septicum, Fusobacterium* spp. and *Escherichia coli*. The potential pro-carcinogenic effects of these bacteria are now better understood [5]. Gut resident bacteria are able to produce a number of metabolites and bioproducts necessary to protect host's and gut's homeostasis. Conversely, several microbiota subpopulations may expand during pathological dysbiosis and therefore produce high levels of toxins capable, in turn, to trigger both inflammation and tumorigenesis [12]. Importantly, gut microbiota can interact with the host either modulating directly the gut epithelium or the immune system. Numerous gut populating bacteria, called probiotics, have been identified as protective against the genesis of tumors. Given their capability of preserving gut homeostasis, probiotics are currently tested to help to fight dysbiosis in cancer patients subjected to chemotherapy and radiotherapy. Most recently, three independent studies show that specific gut resident species may potentiate the positive outcome of anti-cancer immunotherapy [13]. The highly significant studies, uncovering the tight association between gut microbiota and tumorigenesis, as well as gut microbiota and anti-cancer therapy, are here described. The role of the *Lactobacillus rhamnosus GG* (LGG), as the most studied probiotic model in cancer, is also reported [14].

Complex host-microbiota interactions are considered probable primary or secondary contributors to the pathogenesis of CRC. From the microbiota perspective, several hypotheses are actively under investigation, including disease instigation or promotion through individual microbes (model 1), the collective microbiota (model 2), or an interactive model in which single microbes drive the emergence of a modified, disease-generating microbiota (model 3) [2]. From the host perspective, the microbiota may alter tumorassociated inflammation with consequences for tumor biology, or conversely, the tumor microenvironment or associated inflammation may induce microbiota shifts with the potential to further inhibit or promote tumor biology. Host genetic polymorphisms that modify immune and metabolic responses are predicted to play a key role in host-microbiota interactions during colonic carcinogenesis [11].

2.1 Microbial contribution in tumorigenesis of colorectal cancer

Malignant degeneration of intestinal epithelial cells (IECs) and progression to CRC involves a complex interplay from various layers of extrinsic and intrinsic factors. Together these influences result in oncogenic mutations in Lgr5+ intestinal stem cells (SCs), in altered b-catenin/Wnt signaling, and in pro-inflammatory programs that drive CRC [15]. Extrinsic, predominantly nutritional factors can directly damage host DNA, modulate the composition and metabolic activity of the gut microbiota, interfere with gut barrier functions, affect intestinal epithelial cells (IEC) metabolism, and influence immune functions.

The microbiota influences intestinal tumorigenesis through several mechanisms. Several CRC-associated species such as *Fusobacterium nucleatum*, colibactin-producing *Escherichia coli*, and en- terotoxigenic *Bacteroides fragilis* have been implicated in DNA damage and tumor progression [16]. Microbial metabolism of complex carbohydrates, bile acids, and luminal iron, including heme iron, are important for barrier function and immune homeostasis as are bacterial signals integrated by surface and intracellular pattern recognition receptors such as Toll-like receptors, NOD-like receptors, and inflammasomes expressed by various cells types. Chronic inflammation represents an important intrinsic factor that promotes carcinogenesis by inducing DNA damage, and reactive oxygen and nitrogen species, by modulation of IEC polarization and the tumor microenvironment, by activating transcriptional programs such as nuclear factor kB and STAT3 in IECs, and by hampering anti-tumoral immunity [5].

The tumor-associated luminal environment represents a niche characterized by an impaired barrier function and a cluster of commensal bacteria that have been implicated in tumor initiation and progression. For example, *Fusobacterium nucleatum*'s (*Fn*) FadA antigen binds E-cadherin on IECs to activate b-catenin. This leads to uncontrolled cell growth, acquisition of a stem cell-like phenotype, loss of cell polarization, and possibly microsatellite instability (MSI) that is proved in the diversity of types of colon cancer cells between the right and the left colon [17]. By mechanisms including TLR4 and MyD88, *Fn* has proinflammatory effects on the tumor microenvironment. Furthermore, *Fn* has been shown to modulate autophagy in IECs by activating regulatory microRNAs. On the other hand, *E. coli* strains harboring the polyketide synthases (pks) island encoding the genotoxin colibactin are frequently observed in human colorectal tumors. Besides the genotoxic activity of colibactin, cell growth is sustained by cellular senescence associated with the expression of hepatocyte growth factor (HGF) [18].

Theenterotoxigenic *Bacteroides fragilis* (ETBF) causes inflammation and tumors in animal models. ETBF induces spermine oxidase (SPO) generating reactive oxygen species (ROS), thereby inducing DNA damage. ETBF is associated with Th17 responses. Finally, certain sulfate-reducing bacteria such as *Bilophila wadsworthia* or *Alistipes* spp. produce hydrogen sulfide (H_2S) capable of inducing geno- toxic insults. Both strains promote inflammation in susceptible animals [10].

Certain nutrient carcinogens including heterocyclic amines, polycyclic aromatic hydrocarbons, and N-nitroso compounds exert direct genotoxic effects. Intestinal homeostasis and an intact gut barrier function ensure spatial segregation and exclusion of luminal threats. Nutrition may be a key modulator of gut microbial composition and barrier function [19]. A diet deprived in microbiota accessible fiber promotes mucus degrading species and deprivation of short-chain fatty acids (SCFAs). SCFAs strengthen barrier functions through mechanisms such as G-protein-coupled receptor (Gpr)-mediated sensitization of the IEC inflammasomes and reducing IEC oxygen concentrations and induction of hypoxia-induced factor (HIF). Furthermore, SCFAs exert anti-inflammatory and tolerogenic effects on immune cells. Gut barrier function may be further deteriorated by certain food additives including dietary emulsifiers. Diets enriched in red meat and animal fat promote the overgrowth of proinflammatory and protumorigenic species by altering bile acid metabolism. Heme iron further exerts direct cytotoxic and hyperproliferative effects [12].

Commensal organisms within the lumen of the gut have profound influences on the immune system at the local level within the gut mucosa, in draining mesenteric lymph nodes, and systemically. The immune system likewise can alter the gut microbiota. Goblet cells create a thick mucus protective layer covering the mucosa; this mucosal layer is largely deficient in GF animals. Plasma cells in the lamina propria secrete IgA into the lumen of the gut. Paneth cells secrete a number of anti- microbial peptides; their activity is amplified in response to signaling from local immune cells in response to the microbiota [5]. Bacterial metabolites or bacteria themselves can activate local DCs which migrate to the draining lymph nodes to activate naive T cells to effector T cells, Tregs, or Th17 cells, which can migrate back to the gut mucosa or enter systemic circulation. Specific metabolites or bacterial byproducts can alter the DC in a fashion that allows them to skew toward a Treg versus Th17 phenotype. Tregs function in secreting IL-10, creating a local anti-inflammatory cytokine milieu. Th17 cells, meanwhile, produce IL-17, which can increase Paneth cell production of anti-microbial peptides and can function in recruiting polymorphonuclear neutrophils (PMNs) from the bloodstream. Some bacterial metabolites can enter the bloodstream directly further altering the systemic immune system. Metabolism of fiber by colonic microbes results in generation of butyric acid. When genetic mutations in Msh2 and Apc are present, butyrate increases cell proliferation and enhances tumorigenesis. Data from another model of colorectal carcinogenesis indicate the opposite outcome: Neoplastic colonocytes engage in glycolysis for cellular energy, unlike healthy colonocytes (which favor fatty acid oxidation). As a result, butyrate accumulates in the nucleus of neoplastic cells, engaging tumor-suppressive pathways and apoptosis [18].

Composition of the gut microbiota has profound effects on intestinal carcinogenesis. Diet and host genetics play critical roles in shaping the composition of gut microbiota. Whether diet and host genes interact with each other to bring specific changes in gut microbiota that affect intestinal carcinogenesis is unknown. Ability of dietary fibre to specifically increase beneficial gut microbiota at the expense of pathogenic bacteria *in vivo* via unknown mechanism is an important process that suppresses intestinal inflammation and carcinogenesis [20]. Free fatty acid receptor 2 (FFAR2 or GPR43) is a receptor for short-chain fatty acids (acetate, propionate and butyrate), metabolites of dietary fibre fermentation by gut microbiota. Here, we show FFAR2 is down modulated in human colon cancers than matched adjacent healthy tissue. Consistent with this, Ffar2(-/-) mice are hypersusceptible to development of intestinal carcinogenesis. Dietary fibre suppressed colon carcinogenesis in an Ffar2-dependent manner. Ffar2 played an essential role in dietary fibre-mediated promotion of beneficial gut microbiota, *Bifidobacterium* species (spp.) and suppression of *Helicobacter hepaticus* and *Prevotellaceae*. Moreover, numbers of *Bifidobacterium* is reduced, whereas those

of *Prevotellaceae* are increased in human colon cancers than matched adjacent normal tissue. Administration of *Bifidobacterium* mitigated intestinal inflammation and carcinogenesis in Ffar2(-/-) mice. Taken together, these findings suggest that interplay between dietary fibre and Ffar2 play a key role in promoting healthy composition of gut microbiota that stimulates intestinal health [21].

Dietary component also play beneficial roles beyond basic nutrition, leading to the development of the functional food concept and nutraceuticals. Prebiotics, polyunsaturated fatty acids (PUFAs) and phytochemicals are the most well characterized dietary bioactive compounds. The beneficial effects of prebiotics mainly relay on their influence on the gut microbiota composition and their ability to generate fermentation products (short-chain fatty acids) with diverse biological roles. PUFAs include the omega-3 and omega-6 fatty acids, whose balance may influence diverse aspects of immunity and metabolism. Moreover, interactions between PUFAs and components of the gut microbiota may also influence their biological roles. Phytochemicals are bioactive non-nutrient plant compounds, which have raised interest because of their potential effects as antioxidants, antiestrogenics, anti-inflammatory, immunomodulatory, and anticarcinogenics. However, the bioavailability and effects of polyphenols greatly depend on their transformation by components of the gut microbiota. Phytochemicals and their metabolic products may also inhibit pathogenic bacteria while stimulate the growth of beneficial bacteria, exerting prebiotic-like effects. Therefore, the intestinal microbiota is both a target for nutritional intervention and a factor influencing the biological activity of other food compounds acquired orally [22].

2.2 Gut microbiota and colorectal cancer therapy

According to Farrokhi*et al.*, [23], regulatory effects of microbiota have been shown in different types of cancer therapies such as chemotherapy and immunotherapy. Immune-checkpoint-blocked therapies are the recent efficient cancer immunotherapy strategies. The target of immune-checkpoint blocking is cytotoxic T lymphocyte protein-4 (CTLA-4) or blockade of programmed death-1 (PD-1) protein and its ligand programmed death ligand 1 (PD-L1) that they have been considered as cancer immunotherapy in recent years. In the latest studies, it have been demonstrated that several gut bacteria such as: *Akkermansia muciniphila, Bifidobacterium* spp., *Faecalibacterium* spp., and *Bacteroidesfragilis* have the regulatory effects on PD-1, PD-L1, and CTLA-4 blocked anticancer therapy outcome [24, 25].

Interference of the binding of programmed cell death protein 1 (PD-1) and programmed death-ligand 1 (PD-L1) has become a new inspiring immunotherapy for resisting cancers. To date, the FDA has approved two PD-1 monoclonal antibody drugs against cancer as well as a monoclonal antibody for PD-L1. More PD-1 and PD-L1 monoclonal antibody drugs are on their way in clinical trials.

Recent evidence shows there is a bidirectional crosstalk between the gut microbiota and mitochondria. Microbiota and their byproducts (SCFA and secondary bile acids) regulate redox balance and energy production. Secondary bile acid metabolism might also directly modify mitochondrial biogenesis, inflammation and intestinal barrier function in different types of cells [11, 26]. In the mitchondria of colonocytes, butyrate undergoes FAO which produces acetyl-CoA that enters the TCA cycle resulting in ATP and CO_2. Among the SCFA, butyrate is a key regulator of energy production and mitochondrial function by inducing PGC-1α gene expression in skeletal muscles and brown adipose tissue [11] and improving respiratory capacity and FAO via AMPK-ACC pathway activation [27].

Mitochondria regulate gut functions [28], such as intestinal barrier protection and mucosal immune response, which help maintain the mucus layer and intestinal microbiota. *SIRT1* maintains intestinal barrier function through various mechanisms such as enhancing crypt proliferation and suppressing villous apoptosis, stimulating intestinal stem cell expansion in the, regulating tight junction expression of zonulin ocludin-1, occludin and claudin-1 during hypoxia [29]. Mitochondrial genome variants may affect the gut microbiota composition. For example, polymorphisms in the *ND5*, and *CYTB* genes or D- Loop region of mitochondrial genome have been associated with specific gut microbiota compositions like *Eubacterium* and *Roseburia*, which are butyrate producers. Additionally, the European haplotype HV has been associated with decreased odds of severe sepsis, higher OXPHOS capacity and ROS and RONS production as well as elevated VO_2max and aerobic ATP production in response to exercise [30].

In the colon, the gut microbiota ferment indigestible dietary fiber such as resistant starch and oligosaccharides to produce SCFA in the intestines that can account for up to 10% of human caloric requirements [31]. SCFA are key mediators of mitochondria energy metabolism and act as ligands for free fatty acid receptors 2 and 3 (*FFAR2, FFAR3*) that regulate glucose and fatty acid metabolism [32]. SCFA regulate SIRT1 which plays a role in mitochondrial biogenesis via *PGC-1*α deacetylation. In skeletal muscle cells, butyrate phosphorylates AMPK and p38 which then activates *PGC-1*α and thus FAO and ATP production. Butyrate also activates AMPK via UCP2-AMPK-ACC pathway [32]. Commensal bacteria such as *Lactobacillus rhamnosus* CNCMI-4317 has been associated with increased *Fiaf* expression. In

lamina propia macrophages, SCFA also inhibit NF-κB activation that reducing inflammation associated with ulcerative colitis. The result is increased mitochondrial biogenesis, FAO, OXPHOS, oxygen usage, glucose uptake, AMP, ATP ratio and glycogen breakdown and reduced apoptosis [29]. Anaerobic bacteria degrade 5 - 10% of bile acids, and secondary bile acids regulate carbohydrate and lipid metabolism by modulating the transcription factor receptors farnesoid X receptor (*FXR*) and G-coupled membrane protein 5 (*TGR5*) resulting is increased FAO and OXPHOS. FXR mediates carbohydrate metabolism via regulating *SIRT1* and *Fiaf* expression as well as *SREBP-1c* and *ChREBP* activation and fatty acid metabolism via PPAR-α activation [32]. There is increasing evidence that secondary bile acid metabolism might also directly modify mitochondrial biogenesis, inflammation and intestinal barrier function in different types of cells [11, 33, and 34]. The result of SCFA and secondary bile acid's role in mitochondrial biogenesis is better overall athletic performance due to better oxygen uptake, energy availability and fatigue resistance.

The exchange of gut microbiota between individuals has been used to cure pathogens infections or in the treatment of gut inflammatory disease and dysbiosis. For example, FMT has been used to cure recurrent *Clostridium difficile* duodenal infection [35]. Moreover, FMT has been used in a Graft Versus Host Disease (GVHD) after allogeneic stem cell transplantation [36]. Regarding anti-tumor therapeutic applications, preclinical studies performed in mice demonstrated the efficacy of FMT in reducing colon tumorigenesis, although the efficacy in clinical trials still needs to be further proven. Several clinical trials, designed to evaluate the use of FMT in cancer patients are currently ongoing, with the common goal of preventing and/or ameliorating intestinal side-effects of anti-cancer therapies in cancer patients [37]. Despite the success of FMT, there is still a lack of control in this procedure because the whole gut microbiota is transferred along with the therapeutic bacteria species. Therefore, it is of key importance the careful control of the donors' health and their gut microbiota specific composition [38].

3. Conclusions

- The advent of modern molecular microbiota sequencing techniques, has strongly improved the characterization of microbiota variations in CRC. However, a better understanding of the interactions between the host and pathogens in colorectal carcinogenesis requires further microbiota functional studies, especially with respect to metabolomics and RNA sequencing approaches. All of the above mentioned studies published in this regard have been performed without classifying tumors according to their molecular phenotype. Investigations should also consider the heterogeneity of CRC tumors by studying microbiota imbalances in relation to molecular pathways involved in colorectal carcinogenesis, such as chromosomal and microsatellite instabilities or CpG island methylator phenotypes.

- In summary, the role of the microbiota in CRC is increasingly evident and perhaps represents a new approach towards the improved therapeutic management of patients with CRC.That questions the usage of both probiotics and FMT in cancer therapy, either as tools to repopulate cancer patients' damaged intestine or even as proper adjuvants in immunotherapy and other kinds of anti-cancer therapies. Correspondingly, care needs to be pursued as patients are often immunocompromised, therefore it is important to evaluate the specific side effects of administering selected bacterial species to such sensitive individuals.

- In the future, the design of novel experimental trials may undertake a personalized-integrated approach, considering the specific clinical and pathological background of each single patient to be treated, in order to gain only the positive outcomes of probiotics administration and/or fecal transplants, possibly without any harmful side effect.

4. References

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