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COLONIC DISEASE AND THE MICROBIOTA

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Abstract: Microbiota is the set of microorganisms that compose our microbial community. The human microbiota comprises a vast collection of bacteria, viruses, fungi and protozoa that co-exist with us and establish physio-metabolic interactions with our cellular pathways. Qualitative and/or quantitative alterations of the microbiota, termed dysbiosis, has already been associated with a wide range of diseases and conditions, including inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), diverticular disease (DD), colorectal cancer (CRC), asthma, obesity, diabetes mellitus, hypertension, and cardiovascular disease. Microbiota changes in the intestinal lumen stimulate chronic inflammation possibly triggering disease in genetically predisposed individuals. In this review we examined all studies published in the last year in order to provide the newest correlation between specific microbiota alterations and colonic disease, with a focus on future perspectives and strategies based on microbiota modulations.

Keywords: Microbiota, Colon cancer, IBD, IBS, Diverticular disease.

INTRODUCTION

The human microbiota is composed of millions of microorganisms (bacteria, viruses, fungi and protozoa) that inhabits our body and shapes our physiology. The microbiota forms a dynamic community that modulates, with bi-directional interactions, immune, metabolic, endocrine and neurological networks. The microbiota of the gastrointestinal tract is comprised primarily of members of the phyla Bacteroidetes and Firmicutes followed by Actinobacteria and Proteobacteria. Through the production of active metabolites, the microbiota may influence the pathogenesis of many colonic diseases, such as diverticular disease (DD)[1], inflammatory bowel disease (IBD)[2] and irritable bowel syndrome (IBS)[3]. In addition, through influencing host gene expression and epigenetic regulation, the microbiota is also been implicated in colorectal cancer (CRC) pathogenesis[4-6]. Relative abundance of specific bacteria, including *Fusobacterium nucleatum*, *Escherichia coli*, *Bacteroides fragilis* and *Salmonella enterica*[7] were found in fecal samples and tissues from CRC patients compared to health controls. These bacteria, through several virulence mechanisms, are able to establish a chronic inflammatory state that facilitates the carcinogenesis process. A meta-analysis conducted by Wong et al[8] on fecal samples collected from CRC patients, showed a microbiota imbalance in CRC patients compared to healthy controls. In particular, CRC patients seem to harbour a specific microbiota core composed by *B. fragilis*, *F. nucleatum, Parvimonas micra, Porphyromonas asaccharolytica, Prevotella intermedia*, *Alistipes finegoldii* and *Thermanaerovibrio acidaminovorans*[8]. Understanding how microbiota interferes with colonic diseases pathogenesis could allow us to block their progression and prevent complications.

COLORECTAL CANCER, CARCINOGENESISAND MICROBIOTA

CRC represents the fourth most deadly cancer worldwide. In 2019, it was estimated to be the third leading cancer type for both new cases and deaths in the United States[9]. In Italy it is the third most frequent neoplasm in men (after prostate and lung cancers) and the second in women (after breast cancer). Until the early 2000s, an increase of incidence in both sexes was observed with a rising incidence in the young population (before age 50 years). The majority of CRC cases are sporadic (90-95%) with a small minority of hereditary cases (5%). Patients with hereditary CRC syndromes, including Lynch's and familial adenomatous polyposis (FAP) harbor inherited germline mutations and must be included in a strict surveillance program for the CRC prevention. Sporadic CRC occurs from somatic genetic and epigenetic mutations due to modifiable risk factors, such as smoking, alcohol, sedentary lifestyle, obesity, history of IBD, excessive processed, and red meat intake[9,10]. It is possible to distinguish three separate pathways within the carcinogenesis process: the adenoma-carcinoma sequence, the serrated pathway, and the inflammatory pathway[11]. The adenoma-carcinoma sequence is the most frequent and the initiation phase is due to an irreversible genetic damage. The inactivation of the *APC* tumor suppressor genes, found in 80% of CRC sporadic cases, prevents the link between βcatenin and the cytoplasmic domain of E-Cadherin. Excess unbound β-catenin molecules are able to translocate to the nucleus and stimulate the Wnt pathway with subsequent abnormal colonic cells proliferation. In addition, β-catenin stimulates the downregulation of adhesion molecules (E-cadherin, zonula occludens, and cytokeratin) promoting motility and invasiveness[12]. *KRAS* mutations are found in 30% to 40% of CRC cases. K-ras protein constitutive activation leads to the overexpression of Ras/Raf/MAP/MEK/ERK and PI3K pathways. The serrated pathway is driven by *BRAF* oncogene mutations (in 15% of CRC cases) which activates the MAPK pathway with the formation of a hyperplastic polyp that will become a sessile serrated adenoma and ultimately cancer. Finally, the inflammatory pathway, well studied in IBD associated CRC, is identified by a chronic inflammatory state within which dysplastic lesions arise directly (without the benign precursor formation) and, if not removed, it will progress to cancer. Genetic and epigenetic alterations are at the base of the carcinogenesis initiation process and can be influenced by specific microbiota components. Active microbiota metabolites interfere with DNA acetylation/deacetylation and phosphorylation/dephosphorylation activating oncogenes and triggering tumorigenesis[4]. The microbiota modulates digestion, nutrients absorption, produces vitamins and metabolites from non-digestible dietary fiber. Some beneficial gut bacteria protect us fermenting dietary fibers into short-chain fatty acids (SCFAs). SCFAs as acetate, propionate and butyrate play a pivotal role as anti-inflammatory molecules in the colon lumen. In fact, they keep the integrity of intestinal mucosa, cooperate with the immune system for pathogens defense and are one of the main energy source for others resident bacterial species. Butyrate-histones acetylation also controls epigenetic changes acting on the nuclear histone deacetylase (HDAC) with consequent modulation of IFN-γ, TNF-α, and NF-κB genes[13]. Intestinal mucosal barrier alterations might support tumor cells growth and invasion. Once in contact with the cells, bacteria are able to invade them with consequent cellular genome alterations that stimulate the carcinogenesis process. *B. fragilis* is an anaerobe bacterium linked to diarrhea, intra-abdominal abscesses, and CRC. The virulence is given by the presence of adhesive molecules (pili and fimbrie), proteases, neuraminidase, hemolysins and lipopolysaccharide (LPS) a toll like receptors (TLR4)-mediated inflammation. Enterotoxigenic *B. fragilis* (ETBF) produces the BTF toxin, able to damage colonic intercellular junction[14]. The activation of a specific signaling pathway is responsible of E-cadherin cleavage with consequent impairment of mucosal junction barrier. Also, the E-cadherin loss of function determine the Wnt/β-catenin pathway with IL-8 production and activation of anti- apoptosis signaling (mediated by NF-kappa B and COX 2). ETBF and his *bft* gene was found in mucosal biopsies of precancerous conditions as serrated lesions and low grade dysplasia adenomas then CRC patients[15]. Adherent-Invasive *Escherichia coli (AIEC)* and Enteropathogenic *E. coli* (EPEC) are the two major strain associated to colon cancer. Their virulence factors (colibactin genotoxin and the effector protein EspF) induce structural DNA damage and enhances tumorigenesis activity[16]. *Salmonella enterica* inhibits NF-KB signaling pathway and IL-6, IL-12, INF-c and TNF-α secretion across typhoid toxin and AvrA molecules. Also, AvrA activates the JAK/STAT and Wnt/β catenin signaling modulating cellular proliferation and inflammatory response. Another bacteria able to interact with the E-cadherin/Wnt/β-catenin signaling is *F. nucleatum*. *F. nucleatum* is a Gram-negative anaerobic bacterium commonly found in saliva and oral biofilm. Several scholars[17] showed how these bacteria are able to facilitate the tumor growth and invasion in the colorectal submucosa. In the adenoma-carcinoma sequence *F. nucleatum* seems to play a role as a facilitator of the "second hit" of the carcinogenesis process because it uses its Fusobacterium adhesin A (FadA) to directly interact with the extracellular domain of E-cadherin located on the surface of intestinal epithelial cells[18]. E-cadherin is the main cadherin expressed in the colonic crypt epithelium and plays a fundamental role in tissue barrier formation and homeostasis maintenance. Several studies showed that E-cadherin is frequently downregulated or lost in many cancers, including CRC. In fact, the disruption of cadherin-mediated adhesion in the colon surface promotes bacterial translocation with consequent chronic inflammation, cancer

development, and invasion[17]. The evaluation of stool and cytological brushes from patient's colonoscopy demonstrates an higher relative abundance of *F. nucleatum, Corynebacterium, Enterococcus, Neisseria, Porphyromonas* and *Sclegelella* in invasive tumors compared to early CRC lesions that harbors instead *Oribacterium, Desulfovibrio, Clostridiales* and *Lactobacillus*[19]. Mice colonized by *F. nucleatum* showed higher levels of pro-inflammatory genes, such as *Ptgs2, Il1b, Il6, Il8*, *Tnf* and *Mmp3* which facilitate intestinal tumorigenesis through the modulation of the intestinal microenvironment[20]. Tissue from colorectal diverticula, adenomas, tumors and paired normal tissue analyzed with qPCR and 16S rRNA gene sequencing showed a relative abundance of *Prevotella*, *F. nucleatum* and *B. fragilis* in CRC compared to health tissue[21,22]. Detection of *F. nucleatum,* by quantitative PCR, was also evaluated in a panel of non-invasive biomarkers of CRC detection. In a study cohort, including 72 patients scheduled for colonoscopy, Tunsjø et al[23] demonstrated a higher levels of *F. nucleatum* in stool samples of CRC subjects compared with control subjects or individuals with colonic adenomas. Higher levels of *F. nucleatum* and FadA genes were also found in CRC tissues rather than in adenomas suggesting a gradual increase in enrichment of *F. nucleatum* from healthy colorectal tissue to adenomas and CRC[24,25]. In CRC tissue, through molecular network signaling and cellular pathway modulation, *F. nucleatum* was shown to upregulate Anoctamin-1 (ANO1), a channel protein that is linked to tumorigenesis and invasion[26]. Bundgaard-Nielsen et al[22] supported the hypothesis that some specific bacteria could be involved in the colorectal adenoma-tumor transformation supporting chronic inflammation in the intestinal lumen. Analyzing formalin-fixed and paraffin embedded (FFPE) tissue from non-cancerous tissue, adenomas and tumors they found higher density of *F. nucleatum* and *B. fragilis* in tumors compared to adenomas. In particular, *F. nucleatum* was detected in 29.3% of tumor samples and *B. fragilis* in 36.4% of cases. Although the relationship between intestinal dysbiosis and carcinogenesis is well defined, further studies are needed to determine the link between specific microorganism and the alteration discussed in the colon.

MICROBIOTA IN IBS AND IBD

Patients with IBD and irritable bowel syndrome (IBS), two of the most common gastrointestinal disorders, are consistently documented to have a different gut microbiota composition compared to healthy control subjects. IBD, which includes Crohn's disease and ulcerative colitis, affects more than 3.5 million people worldwide. Molecular profiles of host and microbial activity were analyzed by Lloyd-Price et al[27]. In their study they highlighted an increase of *Escherichia coli* with relative decrease of *Faecalibacterium prausnitzii* and *Roseburia hominis* in 132 IBD patients. The study also assessed metabolic profiles of the microbiota during disease activity periods with low levels of butyrate associated with increased disease activity. A comprehensive review of IBD and microbiota changes is published separately within the Year in Microbiota series[28]. Similar microbiota alterations have been associated with IBS. IBS is characterized by abdominal pain and altered bowel habits classified in constipation (IBS-C), diarrhea (IBS-D) or both conditions (IBS-M). Evidence suggests that IBS patients have an increased abundance of *Bifidobacterium, Bacteroides* and Enterobacteriaceae species compared with healthy controls[29]. Furthermore, intestinal dysbiosis not only modulates IBS symptoms but also predicts the long term prognosis. For example, patients with relative abundance of *Lachnospira* and *Clostridium*, showed an increase risk of post-IBS-infections[30]. In order to reduce the adverse effects related to tricyclic anti-depressants, antispasmodics and selective serotonin reuptake inhibitors commonly used in this disease the use of probiotics containing *Bifidobacterium* and *Lactobacilli species* have been shown to be effective in alleviating IBS symptoms compared to placebos validating the hypothesis that microbiota modulation plays a fundamental role in the control of IBS[31]. In addition, implementing a low-FODMAP diet has shown good results for IBS patients[32]. Low FODMAP eliminates the fermentable substances as lactose, fructose and polyols reducing IBS symptoms through the modulation of the osmotic effects of food in the gut lumen[32]. However, does it remain to be established whether the change in microbiota diversity is a cause or an effect of the inflammation in these disease? Further studies are needed to clarify the role of the microbiota in IBD and IBS.

MICROBIOTA AND DIVERTICULAR DISEASE

The increase in the diverticular disease (DD) and diverticulitis prevalence in industrialized countries is mainly due to higher population life expectancy, sedentary lifestyle, obesity, smoking and unhealthy dietary habits[33,34]. Intestinal dysbiosis seems to be involved in the etiopathogenesis of acute diverticulitis. In fact, the relative increase of proinflammatory bacteria intestinal lumen would determine a chronic inflammatory

state that facilitates bacterial translocation across the intestinal barrier. In addition, patients with DD show a decrease of SCFAs producing-bacteria, which are known to physiologically nourish colonic epithelial cells and reduce inflammation. Low levels of *Akkermansia* and higher levels of Proteobacteria are also associated with diverticulitis and its complications including abdominal abscesses and peritonitis. However even though a relationship between the microbiota and diverticular disease has been ascertained, further studies are needed to determine exactly how the microbiota it is involved in this disease.

DISCUSSION

There are multiple lines of evidence supporting the role of the microbiota in the etiopathogenesis of colonic disease. Understanding how the microbiota, together with other modifiable risk factors influence diseases development will open the possibility of new therapeutic strategies that could stop the disease progression, especially in the early stages. For example, dietary intervention reduces intestinal inflammation and improves individual's health representing a low cost and low impact therapies. As it is known, high fat diet and read meat have been associated with intestinal inflammation and carcinogenesis[35]. Colonic fermentation of red meat produces bacterial metabolites that could damages our gut cells. Mutagenic substances as nitrites and nitrates, heme and heterocyclic aromatic amines (HCAs) supporting the peroxidation process directly damage the DNA and stimulate uncontrolled cells proliferation. Microbiota interferes with these intestinal networks and could be used to reduce this dangerous metabolites. Dietary interventions also play a role in preventing diverticulitis in the contest of the combination therapy (high fiber diet, probiotics and aminosalicylates). Also, the use of a high-fiber diet interferes with the composition and metabolism of gut microbiome and increases the production of SCFAs maintaining remission in IBD patients and reducing IBS symptoms as diarrhea, constipation and back pain[36]. Oral supplementation of probiotics rich of *Bifidobacterium* and *Lactobacillus* spp. increases microbial diversity and decreases the abundance of pathogen bacteria as *F. nucleatum* on intestinal mucosal tissue[37].

CONCLUSIONS

Microbiota is strictly involved in the etiopathogenesis of colonic disease, even though the level of its intervention is currently not well understood, and further investigation is required. In the near future, microbiota modulation strategies will represent a new therapeutic approach to reduce the global burden of several colonic disease.

Author Contributions

All authors equally contributed to this paper with conception and design of the study, literature review and analysis, drafting and critical revision and editing, and final approval of the final version.

Conflict of Interest

No potential conflicts of interest. No financial support.

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