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Metagenomic Approaches for Investigating the Role of the Microbiome in Gut Health and Inflammatory Diseases

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Abstract

The human gut microbiota makes fundamental contributions to host metabolism and immune system. Therefore, perturbations in its composition, a process known as dysbiosis, have an important role in the development of several chronicle diseases, mainly intestinal inflammatory disorders. Culture-independent molecular methods are allowing scientific community to uncover substantive findings, thus giving a more detailed description of the human intestinal microbiota. This chapter presents a review on current metagenomic approaches, based on next-generation sequencing technologies, for investigating bacterial taxonomic classification and predictive function associated with the human gut in health and disease. In this context, we describe recent studies that have been trying to elucidate important alterations in microbiome composition across individuals according to delivery mode, aging, diet and medication that might be linked to susceptibility to immune-mediated diseases. A description of the main bacterial taxa and genes acting in dysbiosis during inflammation, focusing on chronic inflammatory bowel diseases and colorectal cancer, is also explored in this chapter.

Keywords: gut microbiota, metagenomics, dysbiosis, inflammatory bowel diseases, colorectal cancer

1. Introduction

Microbiota is considered as an essential organ of humans and other animals, which carry out many functions that host cells cannot. A major portion of these mutualistic microbes is found

in the human gut, where they occupy niches that make contributions to nutrient processing, pathogen colonization resistance and mucosal immune system development [1]. The intestinal microbiome is formed by hundreds of different bacterial species colonizing mucosal surfaces. Its compositional structure differs across human populations according to geographic regions in the world method of delivery at childbirth, breast or bottle feeding, age, diet and medications [2]. Actually, the role of one individual microbiota is composed by the repertoire of expressed genes, known as metagenome. Impressively, it is estimated that humans possess 10 million extra genes from intestinal bacteria [3]. Significant perturbation of the gut microbiota can lead to a dysbiosis state, which compromise important functions in host immunity and raise susceptibility to immune-mediated diseases [4]. Therefore, there has been great interest in identifying the metagenomic content of the gut microbiota which can be used to treat or prevent diseases. In this context, extensive endeavor are being carried out to elucidate the gut ecosystem and molecular mechanisms underlying the pathogenesis of several intestinal disorders [5]. Culture-independent methods, in particular next-generation sequencing technologies, have prompted a huge breakthrough in our knowledge regarding the microbial communities colonizing the human body and their functional beneficence to host health [6].

2. Metagenomic sequencing approaches for investigating intestinal microbiomes

2.1. Bacterial identification by 16S rRNA gene sequencing

New generation sequencing technologies are capable of processing high amount of DNA in a relative short time using 16S ribosomal (16S rRNA) genetic information. Several high through-put platforms such as 454 Roche GS FLX, Applied Biosystems SOLiD System, Illumina HiSeq and MiSeq System and Ion Torrent Personal Genome Machine (PGM) have been used for this kind of metagenomic approach [7, 8]. The molecular-based taxonomic investigation for bacteria employs direct sequencing of PCR-amplified small sequences of 16S rRNA gene from extracted DNA, generally using universal primers annealing conserved nucleotides to amplify one or more fragments of variable regions. As a few numbers of base pairs can change in a very short period of evolutionary time, amplicons around 300 bp are frequently enough for taxonomic assignment [9]. The sequences at a pre-defined level of identity stand for grouped clusters of similar sequencing reads, known as Operational Taxonomic Unit (OTU), which corresponds to a group of very similar 16S sequences. Reference databases (GreenGenes, myRDP, NCBI) are used to classify OTUs providing identification of taxonomy, relative frequencies and diversity of community composition in samples obtained from the certain ecosystem [10, 11]. This approach allows identification of new species and investigation of low-abundance bacteria and even uncultivated gut microbial communities from a single analysis. In addition, these technologies are faster and more accurate compared to classical identification methods (cloning and culture) [12]. However, this approach has some limitations regarding information about the microbiome function, mainly because several species of bacteria have not been characterized yet and secondly due to a great variability found among individuals, it is expected that the microbiota function present high redundancy, in which different species may occupy the same niche in the gut [13].

2.2. Shotgun sequencing for predictive functional analyses

The whole metagenome sequencing can be performed using a shotgun approach. In such genomic survey strategy, multiple continuous overlapping sequences (contigs), which are assembled from fragmented sequences and obtained from total purified genomic DNA, are used for identifying genes through alignment with bacterial reference genomes and databases (KEGG, SEED and NCBI) [6]. Shotgun approach is quite versatile, in which the samples can be submitted to various methods, including nebulization, endonucleases, or sonication for random fragmentation of DNA, sequencing a subsequent contig assembly and annotation. Furthermore, advanced computational methods applying different algorithms are frequently being developed for more accurate assembly and annotation of genes, thus allowing functional characterization in complex environments like the human gut [14, 15]. This method also provides identification of variants and polymorphisms and gives a more comprehensive understanding on the functional information of microorganism communities, for example, by reconstructing metabolic pathways *in silico* [12]. A major limitation of this strategy is that metagenomic sequencing of multiple individuals is extremely expensive in comparison with 16S rRNA sequencing and generation of a large amount of data demands intense computational analysis, most of time to be performed by bioinformatics specialists [16].

2.3. Metagenomic consortium

Massive increase in the amount of data from human gut microbiota and identification of genes or families of genes submitted in the databases has prompted the creation of consortia such as the Human Microbiome Project in healthy individuals, which led to establishment of a reference microbial genome database according to results of 16S profiling of 242 healthy adults from the United States [17]. The European milestone Metagenomics of the Human Intestinal Tract (MetaHIT) intended to identify potential links between the association of gut microbiome with obesity and inflammatory bowel disease (IBD) from 540 Gb of DNA from stool samples of 124 healthy or sick individuals [18]. Moreover, about 1000 bacterial species were found and each individual in this study is estimated to contain at least 160 species, and in addition, 18 species of bacteria were common to 124 individuals [19]. Nevertheless, complementary approaches to metagenomic studies as well as integrative analysis are required to understand the complex and intrinsic interactions with gut microbiota and hosts, like metatranscriptomics, metaproteomics for studying the functional aspects of the microbiota and metabolomics [20, 21].

3. The gut microbiome in health

3.1. Nutritional and metabolic functions

Gut microbiota is essential for the host digestion and nutrition, as they can produce unique nutrients from indigestible substrates [22]. For instance, many polysaccharides, which are found in vegetables from our diet, such as cellulose, xylans, resistant starch and inulin, are digested by certain species that colonize the intestines. Metagenomic studies revealed that the capacity to digest xyloglucans found in onion and lettuce is a specific trait of *Bacteroides* sp. [23]. Through

a mutualistic relationship, the digestion of non-digestible compounds by microbiota may yield energy for microbial growth and end products such as short-chain fatty acids (SCFAs). SCFAs, mainly acetate, propionate and butyrate, are an important energy source for the colonic epithelium and as a key factor for regulating pro-inflammatory immune responses in the gut. Acetate and propionate are important for the liver and peripheral tissues as well, because they act as substrates for metabolic functions such as gluconeogenesis and lipogenesis [5]. The capacity of gut microbiomes metabolic activity, when regarding SCFAs production, may depend on how much complex carbohydrates are ingested through our diet and on the composition of the gut microbiota as well [24].

A study using a model of gnotobiotic mice demonstrated that fermentation of dietary fructans increases when animals colonized with *Bacteroides thetaiotaomicron* are co-colonized with *Methanobrevibacter smithii*. The first species produces more acetate and formate, whereas the second uses formate for methanogenesis, illustrating the importance of interaction between the microbiota organisms to promote nutrient fermentation and absorption and consequently metabolic functions [25, 26].

The ingestion of prebiotics such as inulin can promote expansion of *Faecalibacterium prausnitzii* and *Bifidobacterium* sp. in humans. Both species are important for metabolic functions and immune response regulation in the intestinal mucosa as they produce butyrate and folate, respectively. Folate can be synthesized by the large bowel microbiota and is essential for the synthesis of precursors of nucleic acids, contribute to epigenetic effects, and amino acid metabolism. For example, *B. bifidum* and *B. longum* produce folate in high concentrations [27, 28].

Vitamin B12 is synthesized by different bacteria, for example, *Propionibacterium freudenreichii* and *Lactobacillus reuteri* [29]. Vitamin B12 and its corrinoid precursors play an important role in the gut microbiota as 80% of human intestinal microbes express transporters to capture corrinoids and use vitamin B12 as a cofactor for metabolic pathways. For example, *B. thetaiotaomicron* encodes three vitamin B12 acquisition systems. Folate and vitamin B12 also serve as regulators of gene expression in human gut bacteria and might control genomic interactions between the microbiota and host [30].

3.2. Immune system regulation and resistance against opportunistic pathogens colonization

The mucosal immune system is responsible for maintaining gut homeostasis as it must remain vigilant against pathogen infections while limiting over inflammatory responses against the majority of commensal organisms that comprise the microbiota. Symbiotic bacteria play an important role in preventing inflammatory diseases, however in dysbiosis, opportunistic pathogens may stimulate a local immune response resulting a tissue damage. Therefore, the microbiota has the potential to exert both pro-inflammatory and anti-inflammatory responses, meaning that the balance in the composition of gut microbiome may be intrinsically involved in the proper function of the immune system [31, 32]. Many bacterial species from the gut microbiota such as *Lactobacillus* sp. and *Bifidobacterium* sp. have the ability to activate molecular

mechanism on several cell types constituting the epithelial barrier function, such as specialized epithelial cells like goblet cells and paneth cells [33, 34]. Goblet cells are the main cells involved in mucus production, which is considered the first line of host defense against pathogens. Current studies reveal that several commensal microbes can stimulate Muc2 expression, a major component of the intestinal mucus [35].

Enterocytes, such as paneth cells, which reside at the base of the intestinal crypts, are specialized in producing antimicrobial peptides preventing overstimulation of the immune system by keeping commensal bacteria 50 μm apart from the small intestinal epithelial surface [35]. Studies suggest that expression of these antimicrobial peptides, including RegIIIγ and defensins, is driven by the microbiota. Certain components of the microbiota, including *B. thetaiotaomicron*, confer resistance to *Candida albicans* by promoting the expression of H1F-1α, a transcriptional regulator that induces the expression of the antimicrobial peptide LL-37, with anti-Candida activity [36].

Microbiota induction of the adaptive responses of the immune system, including B cells and T cells, plays a central role in the defense against intestinal pathogens and regulation of inflammation in the gut. Certain intestinal bacterial species play a major role in the differentiation of T cells into different subsets, including T helper cells Th1s, Th2s, Th17s and T regulatory cells (Tregs) [35]. The development and functionality of Tregs in the gut depend on the presence of specific commensal microbes. Administration of a pool containing bacterial strains from the Clostridia clusters IV, XIVa and XVIII has been shown to restore the population of Tregs to those observed in conventional mice. One mechanism by which Clostridia species, including *F. prausnitzii*, may enhance Treg differentiation is through production of SCFA [37]. Similarly, vitamin A metabolite retinoic acid, whose production has been shown to be partly controlled by the microbiota, also supports anti-inflammatory function [38].

Production of IgA by B cells is a key factor for the host to control infections on mucosal surfaces, including the gastrointestinal tract. Several studies have demonstrated that the intestinal microbiota influences B-cell development and antibody production. The gut microbiota affects IgA class-switch recombination in either T-cell–independent or dependent pathways. T-cell–independent produces IgA with low affinity but directed towards the microbiota. Bacteria that colonize the epithelial surface of the intestinal mucosa, such as *Mucispirillum*, *Clostridium scindens* and *Akkermansia muciniphila* which are segmented filamentous bacteria, can activate t-cell-dependent pathway and regulation of mucosal IgA responses [39, 40].

The microbiota can also confer resistance to pathogens by directly inhibiting them, without the involvement of the gut immune system. Certain commensal strains can produce and secrete small molecules with bacteriostatic or bactericidal activity, such as bacteriocins or microcins produced by Gram-positive and Gram-negative species, respectively. SCFA can also influence the expression of virulence factors. For example, butyrate and propionate can downregulate the expression of pathogenicity island 1 (SP1) genes in *Salmonella typhimurium*, which is crucial for this bacterium to invade intestinal epithelial cells. A major mechanism by

which the microbiota inhibits intestinal colonization by bacterial pathogens is through nutrient competition [35].

3.3. Microbiome composition in human ontogeny

The gut microbiome assumes different characteristics regarding diversity, structure and functional gene repertoires over human lifetime (**Figure 1**). From the compositional perspective, microbiota becomes more and more complex with time, along with periods of ecological stability and fluctuation due to new environmental expositions, until it reaches a dynamic equilibrium in adulthood [13].

3.3.1. Birth

It is commonly accepted by scientific community that our first exposition to microbes may occur at birth delivery. However, recent metagenomics studies reveal that non-harmful bacteria may colonize the placenta, amniotic fluid and other fetal components from healthy term pregnancies [41]. The bacterial taxa identified in the fetal environment present low abundance but are diverse, including many species associated with the oral and intestinal microbiomes, such as *Fusobacterium* spp. and *Bacteroides* spp. Moreover, similar bacterial communities can be found in meconium, suggesting that the colonization of the gut may start with intrauterine resident bacteria [42]. Nevertheless, the elucidation of the probable mechanism in which those bacteria translocate to the gut of the fetus is required to sustain the hypothesis of prenatal colonization. Regarding the gut microbiome just after birth, it has significantly lower diversity and higher variability among individuals compared to the microbiome in adulthood [24]. The dominant bacteria at phylum level in the neonatal gut include Firmicutes, Proteobacteria and Actinobacteria with lower levels of Bacteroidetes, a dominant phylum in the adult gut microbiome [43].
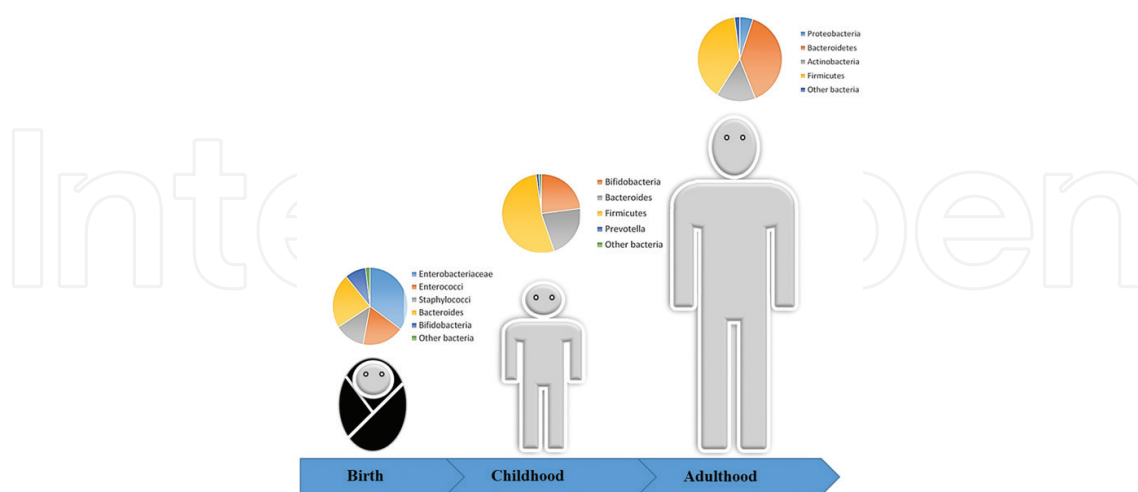

Figure 1. Intestinal microbiota in birth, childhood and adulthood. Birth delivery starts the colonization of the gut, thought the caesarian mode can alter the microbiome composition in newborns. In childhood, gut microbiota shows increased diversity due to introduction of solid foods. In adulthood, the gut microbiota achieves stability but several lifestyle factors may influence the microbiome composition, such as diet, physical activities, alcohol, smoking, drugs and hygiene.

3.3.1.1. Mode of delivery

The modes of delivery significantly influence the neonatal microbiome [44]. In vaginally delivered infants, the gut is primarily colonized by bifidobacteria as well as lactobacilli and enterobacteria. When compared to children delivered in natural terms, the microbiomes of caesarian-delivered individuals show lower diversity and lower abundance of these bacterial taxa [45]. Moreover, infants delivered by cesarean section have more *Escherichia coli* as well as *Clostridium difficile* [46]. A recent study has been trying to evaluate whether cesarean-delivered infants exposure to maternal vaginal fluids are able to colonize them with the natural microbiota that is supposed to be acquired during passage through the birth canal. However, these findings revealed that such inoculation provides minimal effect on the bacterial community of the infants, which presented fewer species and lower levels of *Lactobacillus* spp. and *Bacteroides* spp. comparing with samples originally obtained from vaginally delivered infants [47]. It is important to reiterate that despite of discrepancies between studies in the literature, most of them show that mode of delivery may select certain bacterial groups over other types.

3.3.2. Childhood

Previously, based on classical microbiological studies, it was thought that the human gut microbiome would achieve a stable status during the first 4 years of life. However, contemporary studies using metagenomic approaches have shown a different composition in childhood regarding the human gut microbiota when compared to adulthood [48, 49]. In American children cohorts, a study demonstrated that they present higher frequencies of Firmicutes, Proteobacteria and Actinobacteria and less Bacteroidetes [50]. Moreover, teenager's microbiomes seem to be enriched in butyrate-producing bacteria such as *Alistipes* spp., *B. vulgatus* and *B. xylanisolvens* and *Roseburia* spp., *Faecalibacterium* spp., *Ruminococcus* spp., that seems to play anti-inflammatory roles [51]. Also, on a nutritional function perspective, microbiomes of children contain more vitamin B12-producing bacterial species than adults [52].

Although many functional features of the microbiome of children remain to be profoundly investigated, in general, it is known that childhood microbiome represents a dynamic ecosystem, which probably has an impact on health later in life. In this context, several environmental exposures are supposed to be associated with differences found among individuals. These factors include drugs exposure, contact with domestic animals, hygiene, geography and diet [52]. Metagenomic studies suggest that diet has a determinant role for driving the development of childhood microbiome as it needs to adapt to different conditions of nutrients availability [53].

3.3.2.1. Breast feeding versus bottle feeding

In the first years of life, the human gut microbiome is richer in genes involved in digestion of oligosaccharides found in breast milk, while in later childhood, due to the ingestion of solid foods, the gut metagenome is richer in genes involved in the digestion of polysaccharides and vitamin biosynthesis. Therefore, different microbe exposure in modes of feeding might significantly influence microbiome composition and function [52, 53]. Breast-fed children

show an increase in Actinobacteria and a decrease in Firmicutes and Proteobacteria, whereas bottle-fed ones exhibit more abundance of potential pathogens such as *E. coli* and *C. difficile* [42, 54]. Interestingly, breast milk contains many compounds that might affect the micro-biome composition of the infant gut in a positive way, such as immunoglobulin, prebiotic oligosaccharides and diverse maternal milk microbiota species that continually colonize the infant gut [55]. The majority of the studies show that the composition of the gut micro-biota in breast-fed infants is enriched in aerobic organisms compared to formula-fed ones, which present higher prevalence of anaerobic and facultative anaerobic organisms such as *Bacteroides* sp. Certain *Bacteroides* sp. strains are able to digest milk oligosaccharides, sug-gesting a potential competitive relationship between *Bifidobacterium* sp. and *Bacteroides* sp. in breast-fed infants. Metagenomic findings have been important for providing new advances regarding translational researches such as the development of infant formulas that are more similar in composition and function to breast milk. In this context, Oligosaccharide-enriched formulas have been developed, favoring the colonization of infants gut by greater numbers of bifidobacteria [43].

3.3.3. Adulthood

Healthy adult humans may harbor more than 1000 species of bacteria belonging to different bacterial phyla with Bacteroidetes and Firmicutes being the dominant ones [56]. The microbi-ota of adults can achieve the highest diversity regarding human ontogeny development. There is also considerable variation in the intestinal environment, compared to other sites of the gut, and among healthy individuals. The proportion of each phylum apparently varies according to geographical distribution [2, 57]. For instance, some studies have shown that Firmicutes are more prevalent in adults in rural communities, whereas adults in industrialized societies seem to present higher levels of Bacteroidetes [58]. A higher Firmicutes to Bacteroidetes ratio has been mostly associated with metabolism function and body weight gain, although further investigation is required to shed light on the species-associated role in healthy individuals cohort across different geographical location to understand their influence in leanness/obesity [24]. In general, the gut microbiome in adulthood remains relatively stable through adult-hood, except following perturbations such as pathogen infections, antibiotic drugs or drastic dietary shifts [13]. However, as we age, the gut microbiome is enriched in more traits associ-ated with inflammation and metabolic dysfunction. A reduction in *Bacteroides* spp., *Prevotella* spp. and *F. prausnitzii* and an increase in Enterobacteriaceae have been associated with an overall decrease in the quality of life in old age [51]. In general, the gut microbiome in the elder adults exhibits a higher ratio of Firmicutes to Bacteroidetes when compared to young adults and reduction in symbiotic microbes such as Bifidobacteria and Bacteroides [59].

4. Dysbiosis in the pathogenesis of inflammatory bowel diseases

As described above in this chapter, substantial changes in microbiota may sometimes lead to dysfunction resulting in a dysbiosis state. This process has an important role in diseases involving inflammatory responses in the gut, for example, in inflammatory bowel diseases

(IBDs) such as Crohn's disease (CD) and ulcerative colitis (UC). Moreover, excessive oxidative long-term activity of IBD is associated with colorectal cancer (CRC) (**Figure 2**) [60]. IBDs have been considered as global health problem [61], affecting around 3.1 million people in the USA [62, 63]. CD and UC are chronic diseases characterized by periods of relapse and remission. Clinical symptoms of both diseases are similar to each other; however, UC activity is restricted to the colon while CD may affect any part of the gut, more frequently in the ileum and proximal colon [64].

It has been reported that patients with IBD have a reduced diversity of gut microbiota, characterized by the depletion of commensal species belonging to the phylum Firmicutes and Bacteroidetes [65, 66]. Metagenomic studies have pointed out a decreasing in short-chain fatty acids (SCFAs) metabolism genes such as butyrate, which play an important role in the maturation of regulatory T cells, in the gut microbiota of IBD patients [67]. Moreover, it was observed that patients have a reduction in a number of many SCFA-producing species from Clostridia groups, remarkably *F. prausnitzii* [68, 69]. Microbiota patterns such as the reduction in *F. prausnitzii* have been consistently associated with CD patients, during period of active disease or remission, from different geographical regions including Europe and South Asia and may thus serve as a reliable clinical marker [70, 71]. The reduction in species from *Lactobacillus* and *Bifidobacterium* genus has an important role in IBD pathogenesis as well, as these bacteria can downregulate the expression of key proinflammatory cytokines and chemokines in the gut [70]. In the other hand, opportunistic commensals, such as *Mycobacterium avium* sub. *paratuberculosis*, *C. difficile*, *Ruminococcus gnavus* and enterobacteria, are increased [19]. Commensal Sulfate-reducing bacteria are considered as a key factor in the initiation and maintenance of IBD as these bacteria reduce disulfide bonds of the mucus barrier, thereby allowing exposure of the host cells to pathogenic bacteria and toxins [72]. Therefore, these
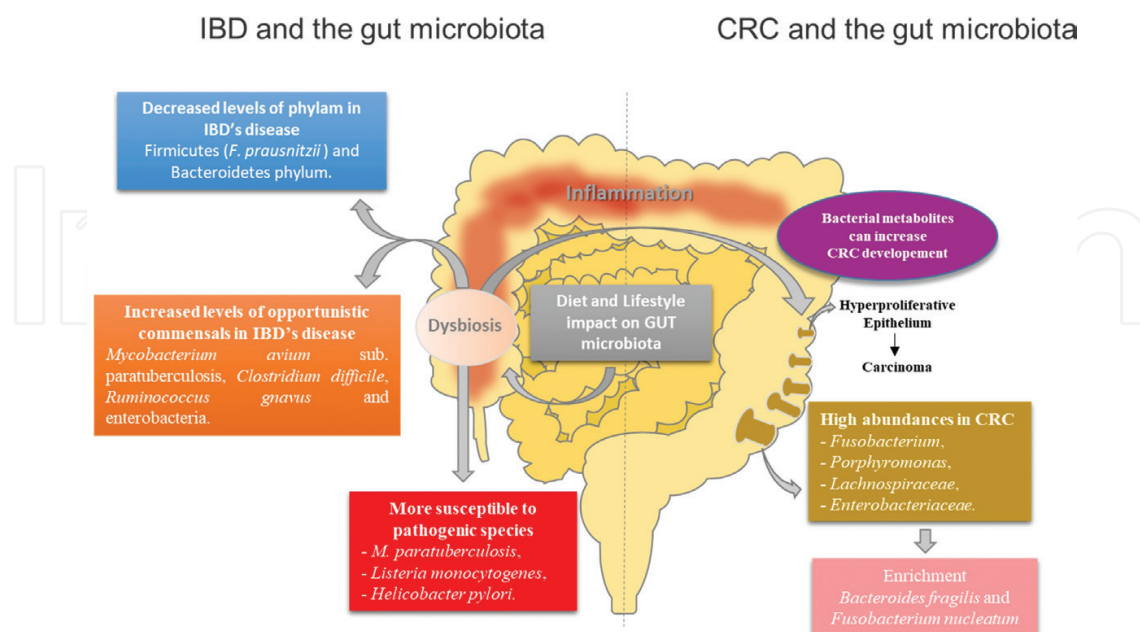

Figure 2. Dysbiosis in pathogenesis of IBD and CRC. Increased levels of metabolites produced by opportunistic commensals of the gut microbiome modulates the effects of inflammation process and cancer tumorigenesis.

patients develop a certain predisposition for the colonization of facultative pathogens, such as invasive *E. coli*, that express several virulence factors involved in adhesion and invasion of the epithelial barrier [73, 74]. Moreover, colonization by pathogenic species, such as *M. paratuberculosis*, *Listeria monocytogenes* or *Helicobacter* species, can worsen the symptoms of IBD as these bacteria can activate proinflammatory signaling cascades into the host [75].

Besides bacteria, gut microbiome fungi and virus may also be involved in IBD. Metagenomic studies demonstrated that inflamed guts present high frequency of certain bacteriophages [76, 77]. In regard of fungi, few studies in mice show some yeast species that can inhibit the over growth of certain opportunistic commensals and affect the gut homeostasis. However, in humans, much research is required to elucidate the role of fungi and virus microbiome in the gut [78].

Currently, there is much discussion about the use of fecal transplant for transference of microbiota from healthy individuals to IBD patients as an alternative therapeutic strategy. Promising outcomes have been achieved against *C. difficile* infection in clinical trials, which is often found in the gut of IBD patients [79]. However, in UC clinical trials, the fecal transplant efficacy is controversial [80, 81]. Regarding CD clinical trials, children cohorts have shown better results with fecal microbiota transplantation than adult cohorts [82, 83].

4.1. Colorectal cancer

CRC is the most common form of gastrointestinal tract cancer, globally the third leading cause of cancer, and is associated with significant mortality affecting both men and women with 1.4 million people diagnosed annually [60, 84].

Different from other kinds of cancer affecting the large bowel which are caused by point mutations in several genes that control cell proliferation, survival, differentiation and migration [60], CRC is mostly attributed to environmental factors and lifestyle such as high fat diet, alcohol, red meat, smoking, obesity, or lack of physical activity [56, 85]. There are several studies showing a connection between inflammatory processes and carcinogenesis, although the contribution of immunological mechanisms and inflammation to malignancy of CRC, it is not fully elucidated [86–88]. Immune cells, cytokines and other mediators of the immune system that are directly influenced by dysbiosis play an important role in the stages of tumorigenesis in the colon, including onset, promotion, progression, and metastasis [86].

Several studies have been trying to identify components of the microbiota that play pivotal roles in inflammation process and in the progression of colorectal cancer by creating microenvironments that favor tumorigenesis development (**Table 1**). Metagenomic analysis of microbiome provides insights of interactions and contributes to understand how the bacterial species can be related to CRC development, as it has been observed that bacterial populations present in fecal samples are distinct from not only tissue biopsies but also between inter-individual microbial communities, even in samples with the same subtypes of cancer. Usually fecal samples and biopsies are collected for 16S RNA gene sequencing generating results about diversity and abundance of the species making part of the gut microbial community [89, 90].

Main findings	Disease	Sampling description	Ref
Microbiomes of IBD subjects fluctuate more than those of healthy individuals	IBD	Fecal samples from 109 patients with IBD (CD, n = 49; UC, n = 60)	[100]
UC and CD have distinct microbiomes. *F. prausnitzii* and *E. coli* were found decreased and increased, respectively, in CD. These species among others could be used as microbiome markers to discriminate CD and UC	IBD	2045 non-IBD and IBD fecal samples from four countries (Spain, Belgium, the UK and Germany)	[101]
Microbiome colonizing the mucosa is different between inflamed subjects with CD and UC. At phylum level, Bacteroidetes is more frequent in CD while Proteobacteria and Firmicutes were more frequently observed in UC. At genus level, *Faecalibacterium*, *Bacteroides* and *Pseudomonas* were significantly different between the inflamed CD and UC	IBD	Analysis of the microbiota composition of ileum, cecum, mid-colon and rectum samples from 166 individuals	[102]
The role of the metabolites produced by the microbiota in dysbiosis was correlated to IBD. Dysbiosis characterized by changes in Firmicutes and Proteobacteria phyla. Decreased levels of *Roseburia* and *Faecalibacterium* were found in CD and UC	IBD	Microbiota of intestinal biopsies and stool samples from 231 IBD and healthy	[103]
Metabolites and fecal microbiome can be useful to discriminate between healthy subjects and patients with IBD. At genus level, *Escherichia*, *Faecalibacterium*, *Streptococcus*, *Sutterella* and *Veillonella* were increased, while level of *Bacteroides*, *Flavobacterium* and *Oscillospira* decreased in IBD group	IBD	Microbiota and the metabolites in stool of 183 subjects (UC—82, CD—50 and 51 healthy controls)	[104]
Mucosa-associated dysbiosis was identified in IBD patients. CD and UC may be distinguishable from the mucosa-associated microbial community structure. CD patients have increased levels of *Escherichia*, *Ruminococcus*, *Cetobacterium*, *Actinobacillus* and *Enterococcus* comparing to controls and UC subjects, and a significant decrease in *Faecalibacterium*, *Coprococcus*, *Prevotella* and *Roseburia* as well	IBD	174 mucus samples from 43 UC subjects, 26 CD subjects and 14 non-IBD controls	[105]
No consistent overrepresentation of potential pathogenic bacteria in CRC tissue. Increased abundance of Coriobacteridae, *Roseburia*, *Fusobacterium* and *Faecalibacterium*. Decreased abundance of Enterobacteriaceae (*Citrobacter*, *Shigella*, *Cronobacter*, *Kluyvera*, *Serratia* and *Salmonella* spp.)	CRC	Resections for primary colon adenocarcinoma of 6 patients	[106]
The gut microbiome could be used as a biomarker for CRC. Adenoma: increased abundances of Ruminococcaceae, *Clostridium*, *Pseudomonas* and Porphyromonadaceae. Lower abundances of *Bacteroides*, Lachnospiraceae, Clostridiales and *Clostridium*. Carcinoma: increased abundances of *Fusobacterium*, *Porphyromonas*, Lachnospiraceae and Enterobacteriaceae. Lower abundances of *Bacteroides*, Lachnospiraceae and Clostridiales	CRC	Analysis of fecal samples from healthy individuals, adenoma and carcinoma patients (30 subject for each clinical group)	[107]
F. nucleatum is prevalent in cases of proximal colon cancer. Amount of *F. nucleatum* increases linearly along the bowel subsites from rectum to cecum	CRC	1102 samples provided from database of colorectal carcinoma cases	[108]

Main findings	Disease	Sampling description	Ref
F. nucleatum, B. fragilis and *F. prausnitzii* could be identified as useful prognostic biomarkers for CRC *B. fragilis* and *F. prausnitzii* correlated with patient's survival in CRC *F. nucleatum* presented higher abundance in non-survival group	CRC	Tissues samples from 108 patients in stages I–IV of CRC with different prognosis	[109]
CRC microbiome is stage-specific and appears to evolve with disease progression. Enrichment of organisms including *Bacteroides fragilis, Fusobacterium nucleatum* and under-representation of *Bacteroides vulgatus, Bacteroides uniformis* and *Faecalibacterium prausnitzii* Enrichment of oral pathobionts in poor prognosis tumors and cancers: *Parvimonas micra, Porphyromonas gingivalis* and *Prevotella* spp.	CRC	Tissue was sampled from 158 CRC patients, 24 adenoma patients and 14 normal colon controls	[110]
Multiple fusobacteria members did not correlate with CRC. Enriched *F. nucleatum, F. necrophorum, Leptotrichia trevisanii, B. fragilis, Parvimonas micra, Peptostreptococcus stomatis* and *Gemella morbillorum* Low levels of *F. varium* and *Cetobacterium somerae* in CRC	CRC	16S rRNA amplicon sequence raw datasets from 12 studies	[111]

Table 1. Recent metagenomic studies in clinical investigation of intestinal inflammatory diseases.

Considering CRC microenvironment and the functionality of intestinal microbiome, it has a high importance as a risk factor or can be directly associated to CRC. Chronic inflammatory processes driven by dysbiosis can affect all stages of tumor development by compounds that can damage DNA, for example, reactive oxygen species (ROS) promoting CRC development [91]. During an inflammatory response, this microenvironment generates ROS and reactive nitrogen intermediates (RNIs) that lead to deleterious DNA promoting carcinogenesis damage or activating pivotal signaling pathways for adenoma formation and growth [86]. In a study investigating CRC in mouse model of tumorigenesis, it was demonstrated that long-term inflammation-mediated breakdown of protective intestinal barriers promotes the production of some inflammatory cytokines such as IL-17 and IL-23 that lead to tumor growth by facilitating bacterial translocation and consequently microbial products that trigger tumorigenesis resulting in adenoma invasion [92]. Blooms of enterobacteria also seem to play a role in CRC as indicated by metagenomic studies of luminal microbiota of inflamed Il10−/− mice reveal that *E. coli* can promote cancer activity modulating tumor development once host inflammation has been established [87, 93].

In a study with fecal samples from 74 patients with CRC, the microbiome was enriched in *Fusobacterium nucleatum* and *Peptostreptococcus stomatis*. Moreover, co-occurrence of these taxa with the other two, *Parvimonas micra* and *Solobacterium moorei*, was found. Interestingly, *P. micra* and the Gram-negative *F. nucleatum* can induce inflammatory responses by binding to lipopolysaccharides from Gram-negative bacteria. Furthermore, *F. nucleatum* was shown to increase intestinal tumorigenesis through recruitment of infiltrating immune cells and through activation of β-catenin signaling [90]. In another study, in which the role of *F. nucleatum* in CRC was investigated by metagenomic analyses of 511 colorectal carcinomas from Japanese patients, it was identified a significant increase in the occurrence of the bacteria [89].

An investigation in 34 patients with four CRC subtypes showed enrichment of Fusobacteria and Bacteroidetes, and decreased levels of Firmicutes and Proteobacteria, including *Porphyromonas gingivalis*, *P. micra*, *P. stomatis* and *F. nucleatum,* create a particular condition by recruiting T cells resembling the immunological aspects in specific colorectal tumors [94]. Prorok-Hamon et al. (2014) have shown, by PCR screening, that from 281 *E. coli* isolates from IBD patients, CRC patients presented increased levels of 21 colonic mucosa-associated *E. coli* strains, with pathogenic traits, including M-cell translocation, angiogenesis and genotoxicity properties [95].

Some works using metagenomics and metabolomics integrative analyses found that enriched levels of Fusobacteria and Proteobacteria create a specific mucosal metabolic microenvironment that was associated with CRC pathogenesis through a large number of chemical and molecular signaling pathways [96–98]. Although fecal microbiome and metabolome may positively correlate with CRC conditions, the role of such interactions is still poorly understood in the pathogenesis of the disease [96].

Helicobacter pylori which well established in the development of stomach cancer since 1994 being classified as carcinogenic by the International Agency for Research on Cancer has also been pointed out as risk factor for CRC, as the infection leads to initial inflammatory response by stimulating IL-1β production, and consequently causing epithelium injury such as metaplasia [85]. In some studies, there was an indication of increased risk of colorectal adenomas by the presence of *H. pylori* due to hypergastrinemia; however, there is a controversy in other metagenomic studies that did not found correlation or even did not identify the presence of the pathogen in the analyzed samples [99].

5. Concluding remarks

These recent metagenomic studies reiterate that microbiome intrinsic factors from particular communities and lifestyle are extremely important and should be considered for future development of novel therapies for IBD. Despite of all efforts to accurately unravel the microbiome role in the gut and its relationship with gut inflammation through metagenomic sequencing approaches, it seems that more complex mechanism might be involved in dysbiosis linked to chronic inflammatory diseases, such as IBD and CRC. In this context, further advances in the area are required to achieve a more precise definition of gut microbiome role, which could allow investments for the search of more effective therapies.

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