Review

Healthy effects of prebiotics and their metabolites against intestinal diseases and colorectal cancer

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Abstract: A specific group of plant and animal oligosaccharides does not suffer enzymatic digestion in the human upper intestinal tract, achieving the colon microbial ecosystem in intact form. The reason for that is their diverse glycosidic bond structure, in comparison with common energetic polysaccharides as starch or glycogen. In this complex ecosystem, these molecules serve as energy sources, via fermentation, of distinctive beneficial bacterial groups, mainly belonging to the Anaerostipes, Bifidobacterium, Coprococcus, Faecalibacterium, Lactobacillus, Roseburia and other genera. The main catabolic products of these fermentations are short-chain fatty acids (SCFA) as acetate, propionate and butyrate, which appear in high concentrations in the lumen around the colon mucosa. Acetate and propionate are associated to energetic purposes for enterocytes, hepatocytes and other cells. Butyrate is the preferred energy source for colonocytes where it controls their cell cycle; butyrate is able to induce cell cycle arrest and apoptosis in tumor colonocytes. These oligosaccharides that increase beneficial colon bacterial populations and induce SCFA production in this ecosystem are called prebiotics. Here, different sources and chemical structures for prebiotics are described, as well as their modulatory effect on the growth of specific probiotic bacterial groups in the colon, and how their fermentation renders diverse SCFA, with beneficial effects in gut health.

Keywords: prebiotics; short chain fatty acids; gut microbiota; acetate; propionate; butyrate; colorectal cancer
1. The Prebiotic Concept

The human gut tract is inhabited by a complex, diverse and highly metabolically active microbial community, called intestinal microbiota that comprises an extraordinary number (up to 100 trillion cells and each person having around 200 prevalent species) of resident commensal bacteria [1,2]. Their combined genomes, known as microbiome, contain more than 5 million genes, thus outnumbering the host’s genetic potential by two orders of magnitude [3]. The microbiota plays a critical role in health and well-being of their host. Gut microbes facilitate nutrients and energy to the host, fermenting and absorbing undigested dietary components but also releasing a variety of metabolites including essential vitamins and short-chain fatty acids (SCFA) that influence locally and/or systemically host physiology [4,5]. At the same time, some intestinal microorganisms have the capacity for harmful effects, via their metabolic outputs and gene products (as H₂S), or potential for pathogenicity [6,7].

Among other important functions, the gut community has a major role in control of epithelial cell proliferation/differentiation, confers protection against invasion by opportunistic pathogens and plays a key role in maturation of the immune system and host metabolism [8,9]. The overall balance of the gut microbial community in terms of its distribution, diversity, species composition and metabolic outputs is relevant in ensuring host homeostasis [5]. There is a large body of evidence linking alterations in the gut microbial composition, and the induced changes in interactions with the host, to several diseases [10], including obesity [11], inflammatory bowel disease (IBD) [12], cardiovascular disease [13], autoimmune arthritis [14] or colorectal cancer (CRC) [15,16]. Therefore, the extent to which the gut microbiota is clinically relevant to human diseases needs to be investigated more deeply.

Nutrient availability is critical in influencing the composition and metabolic activity of gut microbiota [17]. Many studies have shown an increased proportion of bifidobacteria in breast-fed
infants compared to formula-fed infants due mainly to the presence of human milk oligosaccharides. A study over a period of 2.5 years of the gut microbiome in a single infant showed a gradual increase in diversity over time alongside abrupt shifts in the abundance of major taxonomic groups associated to changes in diet or health [18]. The most significant change in microbiome composition and functionality occurs during weaning with introduction to solid foods resulting in a shift within the early 2–3 years of life towards an adult microbiota [19].

Dietary patterns have been demonstrated to affect the structure of the gut microbial community, having consequences in health and disease. Remarkably, some authors demonstrated that the composition of the intestinal microbiota differs significantly between children living in a rural African village and those living in Europe [20]. It has been shown that most diet-driven changes on microbiota occur rapidly and might reverse equally rapidly. Thus, short-term diets, such as those devoid of carbohydrates have been shown to have a pronounced effect on gut microbiome [21]. Shifting from a high-fat/low fiber diet to a low-fat/high-fiber diet caused significant changes in the gut microbiota within 24 h; however, the enterotype identity remained stable [22]. A diet rich in fruit and vegetable fiber has been associated with greater microbial diversity and might exert a positive influence in gut health [23]. In controlled human studies, variations in intake of resistant starch and non-starch carbohydrates induced significant changes in the gut microbiota and these seemed to be dependent of the initial composition of an individual’s gut microbiota [24]. Dietary supplementation with prebiotic fibers (as fructo-oligosaccharides and inulin) and dietary animal reduction can promote the growth of specific groups of bacteria, including bifidobacteria and Bacteroides, reducing simultaneously the relative amount of Firmicutes [6,25]. The presence of probiotics in milk products has been shown also to affect microbiota composition. Thus, higher consumption of fermented milk containing Lactobacillus helveticus in healthy subjects increased Bifidobacterium composition [26].

These relationships between diet and gut microbiomes are not only restricted to humans, as it has been shown that gut microbiome diversities from various vertebrates are adapted to the corresponding species diet (carnivorous, omnivorous, herbivorous). Thus, total protein intake in 33 vertebrate species was associated with more metabolic functions encoded by gut microbiome genes (as proteases), whereas total insoluble dietary fiber was associated with higher diversity in microbial taxons. So, for each giving species, gut microbiome function and structure are associated with dietary intake. As an example, carnivorous gut microbiomes are shifted towards degradation of proteins and amino acids as energy source (conversion of mitochondrial pyruvate into oxaloacetic acid (OAA) devoted for energy generation in TCA cycle); whereas herbivorous ones are shifted towards biosynthesis of amino acids building blocks (generation of more pyruvate and phosphoenolpyruvate from OAA as building block for essential amino acids) [27]. In humans, links have been shown between people consuming more diverse insoluble dietary fiber (that one achieving the colon ecosystem and able to be fermented by this microbial community in order to produce SCFAs) and a healthier status with respect to western lifestyle autoimmune (Crohn’s disease, IBD) or inflammatory diseases (type 2 diabetes, insulin resistance, blood lipids concentrations, CRC, etc.) [28].

It is well known that non-digestible food ingredients or substances are capable of modulating composition and metabolic function of gut microbiota, therefore preventing diverse infectious, inflammatory and neoplastic intestinal diseases [29]. These compounds are called prebiotics and since introduction of this concept, its definition has evolved over the last twenty years. In early studies, prebiotics were defined as “non-digestible food ingredients that beneficially affect host health by selectively stimulating the growth and/or activity of one or a limited number of bacteria in
the colon” [17]. The major beneficial effects of prebiotics seem to occur in the large intestine due to the slow transit of the substrates to be fermented and their effects on microbial diversity, which plays an important role in host health [30].

**Figure 1.** Chemical structures of the different prebiotics families. FOS: fructo-oligosaccharides, GIOS: gluco-oligosaccharides, GOS: galacto-oligosaccharides, HMO: human milk oligosaccharides, IMO: isomalto-oligosaccharides.
More recently, prebiotics have been redefined as “selectively fermented ingredients that result in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health” [31]. The metabolic end-products (as SCFAs) that result from this fermentation have been shown to exert beneficial effects, not only at large intestine but also within the entire human body and/or contribute to the prevention/remission of intestinal or systemic pathologies [32,33]. Indeed, the prebiotic definition is still matter of discussion and recently the concept has been revisited in an effort to shift the focus towards ecological and functional features of the microbiota more likely to be relevant in host physiology [34].

Prebiotics are, therefore, non-digestible oligosaccharides with various origin and chemical properties, differing in chain length, monosaccharide composition, linkage type and branching degree. Although there are several carbohydrates marketed as prebiotics worldwide, there are only a few oligosaccharides for which prebiosis has been clearly demonstrated in human intervention trials. These are inulin, oligofructose (also called fructo-oligosaccharides, FOS), galacto-oligosaccharides (GOS), the synthetic disaccharide lactulose (4-O-β-D-galactopyranosyl-D-fructose) and human milk oligosaccharides (HMO) [34] (Figure 1). Other candidate prebiotics are under investigation; among them, isomalto-oligosaccharides (IMO), arabino-oligosaccharides (AOS), xylo-oligosaccharides (XOS), gluco-oligosaccharides (GLOS), soy oligosaccharides and resistant starch have been regarded as emerging prebiotics that may show similar or improved properties than the market well-established oligosaccharides (Figure 1) [29,39,123].

An aspect common to most of these prebiotic compounds is the fact that they mostly constitute carbohydrate energy reserves from diverse plant species, regardless of their accumulation in seed’s cotyledons (rye), leaves (agave), stems (artichoke), bulbs (garlic), roots (chicory) or tubers (yam) [63,123,161]. In contrast, with the most commonly widespread plant reserve carbohydrate, starch, which can also be found in seeds (maize), stems (sago), bulbs (onion), rhizomes (ginger), roots (cassava), tubers (potato) and fruits (banana) [36,56], prebiotics are not digested by mammalian enzymes responsible for starch degradation and absorption during intestinal digestion. Starch is usually composed of 30% amylose (long linear chains of glucose (linked through α-(1,4) bonds) and 70% amylopectin (tridimensional complex networks of linear gluoses linked through α-(1,4) bonds and branched through α-(1,6) bonds to other linear chains) [9]. Mammals as humans degrade starch into glucose disaccharides (maltose) using the amylases produced from salivary glands and pancreas [4]. Then, the maltases produced at the enterocyte lumen degrade these maltoses, rendering free glucoses which are absorbed at the small intestine [55].

To exert their potentially prebiotic properties, oligosaccharides have to resist the digestive enzymes and survive, at least to some extent, the acidic environment and enzymatic digestion by pancreatic and intestinal brush border enzymes in the upper digestive tract. The oligosaccharides resistance to digestion is associated with several factors, including the identity of their monomeric units (fructose, xylose, galactose, arabinose, rhamnose, etc.), linkages type (as fructose chains linked by β-(1,2) bonds in inulin), as well as their ring form and anomeric conformation [21,68]. Dietary oligosaccharides that escape digestion and absorption in small intestine arrive at the colon and there, they are selectively fermented by a number of different bacterial populations, via competitive and/or cooperative interactions, giving rise to healthy metabolites, mainly SCFA as acetate, propionate and butyrate. Such modulatory effect on gut microbiota has been associated with improvement in overall health, enhancement of host defense mechanisms to gut infections, accelerated recovery of gut disturbances and better absorption of minerals [62].
2. **Structural Diversity of Prebiotics and Biological Activities**

The most widespread prebiotic compounds in nature are formed by fructose chains, where the monomers are linked by glycosidic β-(1,2) bonds. These polymers are called fructans, and their fructose chains may contain or not in one of its ends a glucose molecule. Linear fructan molecules may possess from 2 to more than 60 fructose moieties, calling them fructo-oligosaccharides (FOS) (2 to 10 moieties) or inulin (more than 10 fructose residues, as in chicory (*Cichorium intybus*)) (Figure 1) [21,31]. In about 10% of vascular plant species (like banana, garlic, onion and leek) this is the main energy reserve carbohydrate [48]. Several inulin types occur in nature and they differ in polymerization degree and molecular weight, depending on the source plant, harvest time and processing conditions [39].

Fructo-oligosaccharides (FOS) can be produced either by chemical degradation or controlled enzymatic hydrolysis of inulin by endoglycosidases [49]. Furthermore, FOS can be produced from sucrose at commercial scale, using fungal enzymes [50]. Several studies have shown the bifidogenic effect of inulin and FOS favoring *Bifidobacterium* and *Lactobacillus* growth, and decreasing that of *Bacteroides* and *Clostridium* [51].

Some arabino-oligosaccharides (AOS) from sugar beet pectin [52] and lemon peel [53] promote *Bifidobacterium* growth to the same extent as fructo-oligosaccharides (FOS) and inulin, respectively [54–57], with the highest bifidogenic response obtained with those having higher molecular weight [58]. These compounds are chains of L-arabinose moieties linked by α-(1,5) bonds, branched via α-(1,2) or α-(1,3) bonds with other L-arabinose moieties (Figure 1) [59]. The bifidogenic activity of high molecular weight rhamnogalacturonan I from potato pulp has been reported to be superior to the FOS one [60]. AOS fermentation may diminish the inflammatory conditions in ulcerative colitis (UC) patients. An *in vitro* approach to this has been carried out by fermenting AOS with fecal samples from UC patients and healthy control people. These experiments showed that AOS stimulated bacteria genera as *Bifidobacterium* and *Lactobacillus*, and an increase in SCFA as acetate, which are known to elicit anti-inflammatory responses. AOS may therefore represent a new prebiotic candidate for reduction of flare-ups risk in UC patients in the near future, once *in vivo* tests will be carried out [61].

Xylo-oligosaccharides (XOS), are sugar oligomers made up of xylose units. They are naturally present in fruits, vegetables, bamboo, honey and milk and can be produced at industrial scale from xylan-rich materials (as straw, wood and some macroalgae, where they form the hemicellulose portion). These unusual oligosaccharides are composed by chains of xylose moieties linked by β-(1,4) bonds, with a polymerization degree ranging from two to ten monosaccharides (Figure 1). Several studies have shown the bifidogenic effect of XOS [62,63]. They are considered soluble dietary fibers with prebiotic activity, favoring the improvement of bowel functions and immune function and having antimicrobial and other health benefits as being able to decrease blood glucose levels, total cholesterol and LDL in patients with type 2 diabetes mellitus [64].

Raffinose and stachyose (Figure 1) are non-digestible oligosaccharides present in soybean seeds but also in other legumes like peas or lentils. Raffinose is a trisaccharide containing a galactose moiety linked α-(1,6) to the glucose unit of sucrose. Stachyose is a tetrasaccharide containing a galactose molecule linked α-(1,6) to the terminal galactose unit of raffinose [65]. Deshipustachyose granules is a novel oligosaccharide preparation (55.3% stachyose, 25.8% raffinose and 9.7% verbascose) which promotes the growth of beneficial intestinal bacteria in mice gut, inhibiting
pathogenic bacteria and also facilitating intestinal peristalsis and fecal excretion, thereby enhancing intestinal health and relieving constipation [66].

Resistant starch is present in some plants, where the starch amylose/amylopectin (Figure 1) relation is much higher than the usual 30%/70% respectively. This abundance facilitates the amylose crystallization and compaction processes, and hindering its digestion by amylases along the intestinal tract [67].

Galacto-oligosaccharides (GOS) are naturally-occurring compounds present in mammals’ milk. These prebiotics can be produced industrially from whey lactose by trans-galactosylation of lactose, carried out by β-galactosidases. This fact results in complex mixtures of oligosaccharides with different degrees of polymerization, ranging from two to eight moieties, and different glycosidic linkages: β-(1,1), β-(1,2), β-(1,3), β-(1,4) and β-(1,6) (Figure 1). Appropriate enzymes from different Bifidobacterium species can specifically hydrolyze these β-glycosidic linkages once they arrive at the colon [51]. Recently, the synthesis of lactulose-derived GOS has attracted the attention of the scientific community due to their prospective prebiotic applications, being recognized by their ability to promote the growth of bifidobacteria in vitro (human fecal slurries) [68] and in vivo (rat as a model) [46,69].

Human milk contains, apart from lactose (70 g/L) and GOS (5 g/L), other complex carbohydrates called HMO (human milk oligosaccharides, 10 g/L) that are important dietary factors with multiple functions during breast feeding ages [70,71]. HMO composition is very complex since at least may consist of more than 200 types of combinations of five monosaccharides: glucose, galactose, fucose, N-acetylglucosamine, and N-acetylmuramic acid (Figure 1). Neutral HMO are composed by glucose and galactose but also contain multiple units of N-acetylgalactosamine and fucose. Acid HMO contains, besides the above-mentioned carbohydrates, units of N-acetylmuramic acid (also known as sialic acid). The presence of sialic acid and fucose in terminal positions render these polysaccharides non-digestible by human digestive enzymes. This facilitates its arrival to colon as unmodified carbohydrates, where they promote growth of diverse beneficial bacterial groups. In fact, HMO are responsible for the large numbers of Bifidobacterium present in breast-fed infants’ feces, as they get regular amounts of these prebiotics from mother’s milk [72,73]. In goat’s milk, the presence of other oligosaccharides (OS) similar to those found in human milk has been reported. Among them, i) the existence of neutral oligosaccharides, whose structures are mainly based on lactose with the addition of neutral monosaccharides such as glucose or galactose (Hex), N-acetylglucosamine or N-acetylgalactosamine (HexNAc) and fucose or deoxyhexose (Fuc) and ii) acidic oligosaccharides, containing acidic components such as N-acetylmuramic (Neu5Ac) or N-glycolylmuramic acid (Neu5Ge) [74]. Some of these oligosaccharides, such as those containing fucosyl- or sialyl- groups have been described to have prebiotic and/or pathogen binding activities [75].

Lactulose (4-O-β-D-galactopyranosyl-D-fructofuranose) is a synthetic prebiotic disaccharide composed of galactose and fructose linked by a β-(1,4) glycosidic bond (Figure 1), which can be industrially obtained by chemical isomerization of lactose present in whey permeate [76]. This enzyme-catalyzed synthesis offers new potential for food technology since it can be carried out with crude lactose materials derived from the dairy industry [77]. Lactulose shows bifidogenic properties stimulating the growth of health-promoting bacteria such as Bifidobacterium and Lactobacillus [78]. Lactulose is used not only in nutrition, as prebiotic, but also for treatment of chronic constipation at doses of 10 g/day [79], to maintain blood glucose and insulin levels [80], and to prevent hepatic
encephalopathy in liver failure patients, as lactulose reduces the intestinal absorption of toxic ammonium from lumen, preventing it from reaching the brain [81,82].

Isomalto-oligosaccharides (IMO) are a mixture of short-chain carbohydrates obtained enzymatically from starch. They contain α-(1,6) and α-(1,4) linkages (Figure 1) [30]. While human intestinal enzymes can digest α-(1,4)-glycosidic bonds, α-(1,6)-linkages are not easily hydrolyzed, and therefore they can reach the colon, where they are partially fermented by the microbiota [83]. Different studies have shown that IMO show bifidogenic properties [84,85,86]. IMO can also diminish total cholesterol and triacylglycerides levels in patients treated with hemodialysis [87] and stimulate intestinal and systemic immunity via a shift in the Th1/Th2 balance towards Th1-dominant immunity. Such effects may be due, at least in partially, to the relative increase of Lactobacillus numbers in the gut microbiota of mice [88].

Finally, gluco-oligosaccharides (GLOS) are glucose oligomers with α-(1,6) linkages that can also branch via α-(1,2) and α-(1,3) (Figure 1). These glycosidic linkages are resistant to hydrolysis by mammals’ digestive enzymes [89]. They can be produced from sucrose in the presence of maltose by enzymatic synthesis, or biosynthesized by Leuconostoc mesenteroides. In vitro fermentation studies have shown that they favor Bifidobacterium growth against harmful bacteria [90,91,92].

3. Modulation of Colon Microbiota by Dietary Prebiotics

Once ingested, prebiotics can carry out a selective action for health-promoting taxonomic groups with beneficial metabolic activities [29]. In this sense, prebiotics might favor the growth of Bifidobacterium and/or Lactobacillus species in the colon, considered a major shift in gut microbiota towards a healthier composition. This overgrowth of such beneficial taxons inhibits the uncontrolled growth of undesirable, potentially pathogenic bacteria including Salmonella sp., Campylobacter jejuni, Helicobacter pylori and Escherichia coli, among others [51]. In a pioneered human study to demonstrate their bifidogenic effects, eight healthy volunteers having a controlled diet were supplemented with 15 g/day of oligofructose or inulin, both of which resulted in almost a ten-fold increase in luminal bifidobacteria, which then returned to baseline following withdrawal of the prebiotic supplement [93]. Since then, both prebiotics, and also GOS, have been used in many healthy human trials, with a general significant increase in colon bifidobacteria [94–100]

Intestinal microbiota dysbiosis (an imbalance in body microbiota towards harmful species versus beneficial ones) scenarios are related to diseases as obesity, acting on the harvest and storage of dietary energy [101,102]. Dysbiosis is also a key factor in the development of inflammatory conditions as IBD [103,104] and Crohn’s disease, one of the main forms of IBD, which also shows an increased risk of CRC compared with healthy people [105]. Prebiotics can help in these cases, as for example, daily consumption of 15 g inulin in patients with Crohn’s disease improved disease symptoms and increased gut bifidobacteria [106].

Fecal microbiota diversity is reduced in cases of CRC, where is frequent the presence of opportunistic pro-inflammatory pathogens from Fusobacterium, Campylobacter, Collinsella, Peptostreptococcus, Porphyromonas, Mogibacterium and Anaerotuncus genera, as well as from Enterococcaceae and Erysipelotrichaceae families. In these cases there is also a reduction of Clostridium cluster IV members (as Faecalibacterium prausnitzii and Roseburia), and Bifidobacterium. Some of these taxons have been defined as CRC driver bacteria: those giving an advantage to tumor progression, as production of superoxide radicals (E. faecalis), genotoxic (E. coli)
strains), toxigenic (B. fragilis) or pro-inflammatory compounds (Shigella, Citrobacter or Salmonella). NF-kB transcription factor is one of the main connections between these pro-inflammatory factors and CRC during its early stages, when inflammation induces genetic mutations, inhibits apoptosis and stimulates proliferation and angiogenesis [107].

Also, there is a potential role of the gastrointestinal microbiota in pathogenesis of irritable bowel syndrome (IBS), and in particular in its diarrhea-predominant type, where low numbers of bifidobacteria are found. This fact has suggested that by adding prebiotics in higher amounts in the diet of these patients, their symptoms could remit due to an increase in bifidobacteria populations [108]. A clinical trial using two different doses of GOS patients with IBS found an increase in the number of bifidobacteria in both groups, compared with the placebo group [109].

Apart from bifidobacteria, beneficial attributes are constantly discovered for many other gut bacteria, and there is ongoing debate on extending the range of target beneficial gut microorganisms. Thus, abundant species in the healthy microbiota such as Faecalibacterium prausnitzii, Eubacterium rectale and Roseburia spp. have been reported to produce relatively high amounts of butyrate from prebiotics fermentation, which plays different roles in important processes linked to colonic health, including protection against inflammation and CRC [110].

Furthermore, some studies have shown that CRC is related to variations in the amount of certain species of bacteria in the gut microbiota. For example, it has been reported a detectable increase in the diversity of the Clostridium leptum and Cl. coccoides subgroups in patients with CRC [111]. Differences in bacterial composition between healthy and tumor colon tissue have been described within cancer patients, with some genera of non-pathogenic bacteria, such as Coriobacteria, Roseburia, Fusobacterium and Faecalibacterium overrepresented in tumor tissue [112]. It has been observed that microbiota changes associated to CRC were not only limited to adenomas areas; as normal tissue beside tumors showed also increased levels of Proteobacteria and decreased levels of species in the Bacteroidetes phyla [113]. In other studies, the abundance of Bacteroides and Prevotella genera was higher in cancer tissue of patients than in adjacent normal tissue [114]. This later work is in accordance with a previous study where 15 colon microbial species were associated with an increased risk of cancer (including Bacteroides vulgatus, B. stercoris, Bifidobacterium angulatum and B. longum). In this previous study, higher levels or Bacteroides are expected in diets with high intake of red meat and fat, as both stimulates bile flow, which stimulates Bacteroides growth and are then converted by these bacteria into diverse colon mutagens. In this same study, Lactobacillus acidophilus and others, as well as Eubacterium aerofaciens were associated with a lower risk of the disease, likely due to their high production of SCFA as acetic acid. The presence of other bacteria, as Collinsella aerofaciens, has been also linked to a reduced risk of CRC [115]. In another study, patients with IBD showed lower levels of this species compared with normal subjects. The administration of inulin-type fructans increased levels of Co. aerofaciens, a considered beneficial effect associated with its fermentation [116].

4. Prebiotics Metabolic Effects and Their Catabolism by Gut Microbiome Species

Prebiotics pass along our digestive tract free of enzymatic transformations, but once they arrive at the colon, these oligosaccharides are recognized as a suitable carbon source for diverse probiotic colonic bacteria, such as lactic acid bacteria of the genera Bifidobacterium and Lactobacillus, which generate large amounts of SCFA after their fermentation [117,118], mainly lactate, pyruvate, acetate,
propionate and butyrate, together with significant amounts of carbon dioxide and molecular hydrogen; and at lesser extent, formate, valerate and caproate (the former three accounting for less than 5% total SCFA). As an example, with a daily prebiotic fiber intake of 10 g, about 100 mmol of SCFA can be generated in the colon [49,119].

Butyrate:propionate:acetate relative colon molar concentrations are 1:1:3. Butyrate is the most important SCFA in human health, as it is the preferred energy source of colonocytes, shows anti-inflammatory activity and regulates gene expression, differentiation and apoptosis in host colon cells. In contrast, acetate and propionate do not show a strong antitumor effect [120].

Propionate is readily absorbed by enterocytes, passing to the portal vein circulation, and being taken up by the liver for gluconeogenesis purposes. Acetate is also rapidly absorbed, and distributed by peripheral blood to all tissues, where it is metabolized [121].

Colon dysbiosis triggers alterations in the gut barrier function, allowing translocation to portal vein of bacterial components as lipopolysaccharides (LPS) and bacterial cells. Once distributed to other tissues, these pro-inflammatory factors promote local inflammation in liver and adipose tissue, giving rise to metabolic changes that may derive in obesity, metabolic syndrome and type 2 diabetes. These changes include higher circulating levels of triglycerides and free fatty acids, higher blood cholesterol levels, and insulin resistance in diverse tissues (muscle, liver, etc.). A first relationship between SCFA and metabolic syndrome derives from the observation that low SCFA blood concentrations have been associated to obesity status in laboratory animals and humans. SCFA can increase leptin levels in adipose tissue, a satiety hormone. At gut level, SCFA induce also production of GLP-1 hormone (glucagon-like peptide-1) after binding to GPR43 receptor. This mechanism increases insulin sensitivity. SCFA binding to gut GPR41 receptor induces PYY hormone (peptide YY) liberation. These two hormones act at the brain level by increasing satiety being at reduced level in obese individuals [119,122].

Butyrate induces lipolytic activity in human adipocytes and in animal adipose tissue, mainly through lipoprotein lipase (LPL) transcription activation. SCFA bind to GPR43 receptor also in adipocytes, suppressing insulin signaling and therefore inhibiting fat accumulation and obesity [119,122].

Acetate and propionate (the two main circulating SCFA) inhibit lipid accumulation in non-adipose tissues, as muscle, liver and pancreas. This inhibition helps in reducing side-effects (as local inflammation, insulin resistance and metabolic syndrome) caused by high levels of circulating fatty acids and triglycerides under metabolic syndrome circumstances. This effect seems to be associated with an increased fatty acid oxidation in these organs, and to the use of propionate for liver gluconeogenesis instead of using it for lipogenesis [119]. Acetate serum levels increase cholesterol biosynthesis, which is, however, inhibited by serum propionate [121].

SCFA increase the numbers of regulatory T cells, an anti-inflammatory cells with reduced numbers in obese individuals. Butyrate, for example, inhibits HDAC 6 and 9. This promotes histone H3 acetylation and expression of FoxP3 transcription factor, involved in development and function of these anti-inflammatory cells. This regulation on regulatory T cells may be of interest also in diseases as ulcerative colitis. Propionate regulates against allergic inflammations, hematopoiesis in the bone marrow and the proper function of dendritic cells. Also, SCFA improve epithelial barrier function and gut permeability, by modulating mucin and tight junction proteins expression [119,122,123].

Most butyrate producers are able to degrade complex polysaccharides towards monosaccharides
and glucose. For example, *Roseburia intestinalis* is a good colon xylan-degrading Firmicutes [124]. *Roseburia* is also a good producer of amylase for starch degradation, and some species can also degrade fructans as inulin, as in the case of *R. inulinivorans* and *F. prausnitzii* [125]. Fucose can be also a good metabolic substrate for fermentation by *R. inulinivorans*, in this case leading towards propionate formation instead of butyrate [126].

From these initial monomeric hexoses and pentoses, glycolysis pathway generates pyruvate, which is the substrate of pyruvate dehydrogenase decarboxylation, rendering acetyl-CoA [127]. Acetyl-CoA is then transformed into butyryl-CoA using diverse intermediates (acetoacetyl-CoA, 3-hydroxy-butyryl-CoA and crotonyl-CoA) [110,128]. Starting from this acetyl-CoA, two main catabolic pathways, with no phylogenetic relationship, are involved in butyrate generation from prebiotic compounds in colon bacterial species. The most common one is the route using butyryl-CoA:acyetyl-CoA transferase (BUT) as the final enzyme, generating acetyl-CoA and butyrate from acetate and butyryl-CoA precursors. This enzyme is present in many families belonging to the phylum Firmicutes, as *Roseburia* spp., *Anaerostipes* spp. (family *Lachnospiraceae*), *Eubacterium.rectale*, *Eu. hallii* (family *Eubacteriaceae*) and *F. prausnitzii* (family *Ruminococcaceae*). Also, other phyla share this pathway for butyrate production, as Actinobacteria, Bacteroidetes, Fusobacteria, Proteobacteria, Spirochaetes and Thermotogae [129–133].

An alternative pathway uses as final step for production of butyrate the enzymes phosphotransbutyrylase (PTB) for converting butyryl-CoA in butyrylphosphate and butyrate kinase (BUK) for transforming this in butyrate. However, this second pathway is very uncommon in human colon microbiota species, and has been identified for example in the Firmicutes bacteria *Eu. ruminantium* (family *Eubacteriaceae*), *Coprococcus eutactus* (family *Lachnospiraceae*), *Cl. perfringens*, *Cl. beijerinckii, Cl. acetobutylicum, Cl. butyricum, Cl. eutactus* and *Cl. lituseburense* (family *Clostridiaceae*) [134]. Interestingly, BUT pathway is more common in gut microbiota from omnivores and herbivores, whereas BUK route is more frequent in carnivores [135]. Also, BUT pathway seems to be more effective under colon conditions, where acetate concentrations are usually high (more than 30 mM) [127].

SCFA biosynthesis is usually a complex phenomenon, as these taxons usually collaborate to degrade insoluble prebiotics towards SCFAs after several rounds of enzymatic transformations, as colon syntrophic consortia. For example, inulin is fermented by *Bifidobacterium*, generating acetate. Then, this SCFA, together with the preexisting endogenous lactate, is used by *Clostridium* cluster XIVa and *Faecalibacterium prausnitzii* to produce butyrate. Another example is *Roseburia inulinivorans* and *Faecalibacterium*, which can collaborate to generate butyrate from inulin. Starch is degraded by *Bacteroides* spp., producing acetate and succinate, which is useful for other species as propionate precursor. Xylane is degraded by *Roseburia intestinalis*, producing H₂ and CO₂, which are converted by *Ruminococcus hydrogenotrophicus* into acetate [136].

5. **Prebiotics as Effectors in CRC Prevention**

CRC is the most common cancer (35 cases per 100,000 hab) in western populations, and an important cause of death, after coronary heart diseases and lung cancer, followed by breast cancer [137]. Human populations with lower consumption of saturated fat and red meat, and higher levels of fruits and vegetables (good sources of prebiotic fibers leading to SCFA) show lower CRC incidences [138–141]. This has been also proof in animal models for CRC [142], as in murine
models treated with colon mutagens, the number of tumor lesions in colon mucosa decreased in rats fed inulin, most probably due to the production of butyrate [143,144].

CRC initiates in the colonic mucosa crypts, which cover this surface completely. These crypts are tubular invaginations formed by structured cell layers [145]. In the bottom of the crypt, stem cells are in charge of carrying out the processes of continuous cell division. This is necessary to maintain the structure and function of intestinal epithelium. Division of these stem cells gives rise to more stem cells or to already differentiated progenitor cells of the different cell types present in the colon. These progenitor cells are located in the intermediate area of the mucosal crypts, and their division and differentiation gives rise to differentiated cells found at the top of each crypt and the entire surface of the colonic mucosa. If one of these stem cells at the bottom of a crypt suffers mutations towards uncontrolled growth, their descendants will expand throughout the crypt, replacing normal stem cells and the rest of normal progenitor cells. This will eventually lead to an aberrant crypt, then to a microadenoma and to a large adenoma (polyp); and finally to an adenocarcinoma with metastasis. The entire development process can take over 30 years [146,147].

The most frequent mutations associated with CRC are those affecting cytoplasmic levels of β-catenin, a protein that normally is bound to E-cadherin in complexes. Free cytosolic β-catenin gets bound to ubiquitin in the Wnt-APC complex and is degraded by the proteasome. Defects in APC (very common in CRC cells) cause an increase in free cytosolic β-catenin, which is translocated to the nucleus, where it binds to transcription factors, activating cell proliferation, maintenance the pluripotency of cancer stem cells, increased motility and metastatic capacity [102]. The apc gene is mutated in many familial versions of CRC, as in FAP (familial adenomatous polyposis), and also is an acquired mutation in advanced steps of CRC transformation. Mutations in Mismatch Repair System genes (MLH, MSH2, MSH6 and PMS2) are present in patients with Lynch Syndrome, another familial version of CRC [148].

A 33% of CRC cells possess mutations causing permanent KRAS activation. This membrane GTPase therefore get independent from upstream growth factors signaling pathways and activates constantly other downstream proteins involved in cell growth, cycle progression at the G1/S phase, metabolic changes towards anabolism, angiogenesis development, cell immortalization and metastasis [149,150].

50%–80% of CRC cells show overexpression in MET (the hepatocyte growth factor receptor), which then activates constitutively other cell signaling pathways involved in motility and metastasis (as MAPK and PI3K-AKT-mTOR pathways) [151]. Other membrane receptors, as the human epithelial growth factor receptors HER1, HER3 and HER4 are overexpressed in 35%–89% of CRC cells, giving rise to poor prognosis, lower survival and metastasis respectively. Their activation in CRC induces also MAPK and PI3K-AKT-mTOR cell multiplication pathways [152,153,154].

Several studies suggest that butyrate is able to modulate the activity of NFkB (and some cytokines as TNF-α), reducing the severity of colon inflammation associated to these transcription factor [155]. Butyrate inhibits specifically NFkB translocation to nucleus (which usually takes place once TNF-α activates it). This exerts a potent anti-inflammatory effect. A low inflammatory status has been also associated to lower risk of CRC in several human studies [156,157,158].

In CRC cells, several anti-apoptotic factors, as BCL2A1, block BAK, inhibiting the apoptosis cascade; butyrate is also an inhibitor of BCL2A1 and other relatives [159]. In addition, CRC cells treated in vitro with butyrate overexpressed enzymes involved in the defense against genotoxic and mutagenic agents, which involves a protective effect of butyrate also at the level of detoxifying
enzymes [160,161].

At the energetic metabolism level, butyrate is the main energy source for normal colonocytes, accounting for about 70% of all energy intake. At low concentrations and under reduced cytoplasmic levels of glucose and pyruvate (as in the crypts) butyrate acts as a normal growth and promoter for colonocytes [162,163]. In normal colonocytes, this intracellular butyrate concentration is maintained low because it is rapidly processed by \(\beta\)-oxidation in the mitochondria, as the preferred energy source.

However, in CRC cells, glucose is the main energy source. In these tumor cells, glucose uptake is increased about 10 times, due to overexpression of GLUT transporters and the Warburg effect taking place as in most of cancers. These cancer cells showing Warburg effect have increased levels of cytosol glycolysis, rendering huge amounts of cytosol pyruvate, which, instead of following the canonical aerobic oxidation process in mitochondria (Krebs cycle) is fermented anaerobically in cytosol to produce large amounts of lactate. This excess of glucose in CRC cells displaces butyrate as the main source of energy, and being butyrate accumulated in the CRC cell nucleus, causing histones hyperacetylation (as butyrate is a strong histone deacetylases (HDAC) inhibitor) and leading towards apoptosis induction, which proceeds via the intrinsic/mitochondrial pathway [163–167]. Histones acetylation is one of the main regulatory mechanisms to modulate genetic expression, not only in colonocytes but in other cell types. This acetylation alters the accessibility to DNA transcription, which is important in the processes of tumor formation [168]. Histone acetylation levels are dependent on histone acetyltransferases (HAT) and HDAC. Acetylation of histones H3 and H4 neutralizes the positive charges in their lysine residues and disrupts the nucleosome structure, enabling DNA unfolding and a more relaxed chromatin structure; allowing access of transcription factors and activation of pro-apoptotic genes [169,170]. Interestingly, HDAC inhibitors, as butyrate, appear to be selective within the regions in which gene expression is altered; as in CRC cells [171].

Histone acetylation by butyrate (due to this nuclear HDAC inhibition) causes an increase in p21 expression. p21 is an inhibitor of cyclin dependent kinases (CDKI) that plays a crucial role in cell cycle arrest at the G1/S phase in transformed cells and prevents tumor progression [172]. At the same time, the pro-apoptotic protein BAK is upregulated in the presence of high butyrate concentrations, and this protein is an essential issue for the apoptotic cascade in CRC cells. Both BAK and p21 upregulation is caused by a less efficient binding of the Sp1 transcription factor to their promoter regions, allowing binding by Sp3 (coupled to HDAC1 and HDAC2) [170]. Sp3 then activates transcription of genes involved in blocking cell cycle at G2/M interphase, and also involved in apoptosis induction (cytochrome C release from mitochondria, formation of APAP-1 complex, caspase 9 activation, etc.) [173,174].

Based on this, a few studies have been carried out in order to demonstrate if prebiotic consumption is able to reduce surrogate markers for CRC in humans. One study analyzed if consumption of prebiotic inulin (6 g, twice per day) caused a reduction in pre-neoplastic lesions in colon mucosa (aberrant crypt foci), but failed demonstrating a statistical reduction in this [175]. However, the European SYNCAN study showed a reduction in DNA damage and in fecal water genotoxicity in patients (which were actually surgically operated of previous colon polyps) receiving a symbiotic (prebiotic inulin plus two probiotics, \(B.\ lactis\) Bb12 and \(L.\ rhamnosus\) GG) [176]. Also, a reduction in genotoxic bile acids was observed in another human trial using oligofructose during a period of three months [177].
6. Conclusions

Dietary intake of prebiotics has a major influence in the healthy status of the consumer. These nutraceuticals can be of plant origin (FOS, inulin, AOS, XOS, raffinose, stachyose, resistant starch), of animal milk origin (GOS, HMO), or generated by enzymatic industrial processes (lactulose, IMO, GlIOS). Prebiotics are currently being extensively investigated by using a wide range of in vitro and in vivo approaches, not only as modulators of gut microbiota but also for their effects in a number of clinical conditions including CRC, intestinal disorders like ulcerative colitis, IBD and irritable bowel syndrome, prevention of obesity and constipation. The action mechanism/s by which these dietary oligosaccharides exert beneficial effects relays on their stimulation of the growth of colon probiotics and beneficial taxons with many anti-inflammatory capacities as well as in their catabolic products, especially butyrate, which is able to carry out a diverse and complex regulation of cell cycle in tumor colonocytes, leading towards their apoptosis.

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Conflict of Interest

Authors declare no conflict of interest in this paper.

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