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GUT MICROBIOTA, PROBIOTICS AND COLORECTAL CANCER: A TIGHT RELATION

A. PINO[1], M. DE ANGELIS[2], M. CHIEPPA[3], C. CAGGIA[1], C. L. RANDAZZO[1]

[1]Department of Agricultural, Food and Environment, University of Catania, Catania, Italy
[2]Department of Soil, Plant and Food Sciences, University of Bari, Bari, Italy
[3]National Institute of Gastroenterology "S. de Bellis," Institute of Research, Castellana Grotte, Bari, Italy

Abstract – *Objective:* *Colorectal cancer (CRC) is one of the most frequently diagnosed cancers worldwide. Scientific evidence suggests a relationship between gut microbiota and colorectal cancer occurrence and development. In addition, recent findings corroborate the assumption that probiotics administration could represent a valuable adjuvant therapy to manage gut dysbiosis and to prevent side effects of anticancer therapies.*

Materials and Methods: *A review of the literature concerning the role of gut microbiota, microbial metabolites and probiotics in CRC prevention and treatment with a special emphasis on the mechanism of action and evidence on both animals and humans was conducted. PubMed/Medline, Google Scholar, EMBASE, and the Cochrane Library supplemented with ScienceDirect.com (Elsevier), Wiley Online, SpringerLink, and Cambridge Journals were used as search engine and browsers. None language restriction was applied, and all studies published up to November 2019 have been considered.*

Results: *The analysed data showed that both gut microbiota and microbial metabolites play an important role in CRC occurrence and development. In vitro and in vivo studies suggest that probiotics exert intraluminal and systemic colorectal cancer-preventative effects. In addition, human clinical trials revealed that probiotics have inhibitory effects on the development of cancerous and precancerous lesions along with features to manage cancer treatment side effects.*

Conclusions: *More in-depth studies should be carried out in order to better understand the interactions between host and pathogens correlated with colorectal carcinogenesis. Even though the in vivo results demonstrate the beneficial effect of probiotics in alleviating the anticancer therapies side-effects, further randomized double-blind, placebo-controlled clinical trials are strongly required to fully understand the probiotics' action and to recommend their routine use as adjunctive therapy for CRC prevention and treatment.*

KEYWORDS: *Dysbiosis, Bacterial biota, Metabolites, Post-operative complications, Gastrointestinal side effects.*

INTRODUCTION

Colorectal cancer (CRC) is the third most commonly diagnosed cancer worldwide and the fourth most common cause of oncological death, becoming a global public health problem associated with social and economic burdens[1,2]. Growing evidence suggests a tight relationship between the gut microbiota dysbiosis and the CRC initiation and progression as well as a central role of the gut microbiota in defining both efficacy and toxicity of chemotherapeutic agents[3-5]. A serious paradox exists in treatment strategies for CRC because the cytotoxic effects of chemotherapeutics on gut microbes can further exacerbate any dysbiotic state rather than correct it, with serious implications for drug toxicity and side

Corresponding Author: C.L. Randazzo, Associate Professor; e-mail: cranda@unict.it

effects. A common adverse effect of chemotherapy, resulting in morbidity and mortality, is gastrointestinal (GI) toxicity in the form of mucositis, causing nausea, bloating, vomiting, abdominal pain, and weight loss. This often leads to dose-limitation, which reduces the efficacy of anticancer treatment[6]. In addition to the multiple host pro-inflammatory and apoptotic pathways activated by chemotherapy, the gut microbiota has a central role in both response to cancer therapy and susceptibility to toxic side effects and a critical role in the development of treatment strategies to prevent life-threatening complications and to improve quality of life. Therefore, it is reasonable to implement actions focused to strengthen/restore the gut microbiota homeostasis[6,7]. Accordingly, there is a rising interest in probiotics use as an adjuvant therapy to modulate gut microbiota and to prevent the aforementioned side effects. The therapeutic potential of probiotics has been proven to be effective in treating a variety of medical conditions including both GI diseases and extra-intestinal illness[8,9]. In oncology, probiotics are emerging as a new class of pharmacotherapeutics that could be effective in cancer treatment to manage gut dysbiosis and to prevent life-threatening complications. Especially in CRC patients treated with chemotherapy, there is a good rationale to use probiotics as an adjunctive anticancer therapy. Based on the available data, it is possible to assume that probiotics might serve as a safe and effective adjuvant therapy to limit chemotherapy-related toxicity and side effects, to improve the integrity of the gut mucosal barrier and to decrease infectious complications in surgical CRC patients. These effects are related to the ability of some probiotic strains to modulate both gut microbiota and immune system, to reduce bacterial translocation, to enhance gut barrier function, to exert anti-inflammatory, anti-pathogenic, anti-proliferative or pro-apoptotic activities[10,11]. According to that, the present review was aimed to highlight the relationship among gut microbiota, microbial metabolites and CRC as well as to evaluate the potential of probiotics in CRC prevention and treatment.

MICROBIOTA AND COLORECTAL CANCER OCCURRENCE AND DEVELOPMENT

CRC development has been among the first neoplastic lesion associated with chronic inflammation[12]. Recently, the persistence of gut microbial dysbiosis, even in patients achieving complete remission, has been identified as a possible reason for frequent irritable bowel desease (IBD recurrence and persistence risk of CRC[13,14]. Gut microbiota dysbiosis, beyond lifestyle, genetic predisposition, dietary and environmental factors, could be responsible for CRC occurrence and development in relation to virulence factors, bacterial metabolites or inflammatory pathways. Scientific evidence suggests the existence of a strong link between intestinal microbiota and CRC highlighting that pathogenic bacteria play an important role in colorectal carcinogenesis. As reported in Table 1, metagenomics analysis of faecal and tissue samples revealed significant differences between CRC patients and healthy control. Based on the available information it is not possible to recognise a specific bacterial population or bacterial genera and species under or over-expression as responsible for increased cancer susceptibility and development. Nevertheless, *Bacteroides fragilis*, *Enterococcus faecalis*, *Streptococcus bovis*, *Escherichia coli*, and *Fusobacterium* spp. are suspected to be involved in colorectal carcinogenesis. In particular, *F. nucleatum* has been recently emerged as a potential candidate for CRC susceptibility acting at the early steps of colorectal carcinogenesis promotion. Indeed, Viljoen and co-workers[15] identified a positive correlation between *F. nucleatum* and CRC in advanced stage (III-IV). In particular, it was assumed that *F. nucleatum* uses the FadA virulence factor to adhere and to invade cells[16], thereby activating β-catenin signalling pathway and promoting CRC[17]. Several studies highlighted the existence of an indirect association between *S. bovis* and colorectal carcinogenesis even the exact mechanism involved is still unclear[18-21]. As suggested by Boleij and Tjalsma[22], *S. bovis* beyond gaining a competitive growth advantage in a tumor microenvironment, by using tumour metabolites as a nutritional source, can induce inflammation and/or pro-carcinogenic pathways leading to tumour progression[22]. The involvement of *B. fragilis* in colorectal carcinogenesis has been explained by the presence, in some enterotoxigenic strains, of the *bft* gene encoding the *B. fragilis* toxin (BFT) which directly affects pathways that lead to increase cell proliferation, epithelial release of pro-inflammatory effectors, and DNA damage in *in vitro* studies and *in vivo* CRC-predisposed mouse models[23-27]. The mechanisms linking *E. faecalis* to colorectal carcinogenesis remain still unclear even if the production of ROS has been described in cellular and animal models[28,29]. Moreover, *E. faecalis* can trigger colitis, dysplasia and CRC in a susceptible interleukin (IL)-10$^{-/-}$ mouse model[30]. Although *E. coli* is a commensal bacterium of the GI tract, several studies have demonstrated a clear link between mucosa-adherent *E. coli* and CRC[31-33]. In fact, some CRC-associated *E. coli* strains, thanks to acquired virulence factors, such as the *afa* and *eae* adhesins, are able to adhere and invade the intestinal epithelium[34,35].

TABLE 1. Human clinical trials investigating faecal, cancer tissue and mucosa-adherent microbiota in CRC patients.

Sample type	Patient groups	Method	Outcome: variation in CRC compared to HC		Reference
Faecal samples					
	CRC (*n*=20) HC (*n*=17)	Real-time polymerase chain reaction	*E. faecalis*	↑	Balamurugan et al[38]
			E. rectale and *F. prausnitzii*	↓	
	CRC (*n*=60) HC (*n*=119)	454 pyrosequencing of the V3 and V4 regions of the 16S ribosomal RNA gene; real-time qPCR	*Bacteroides/Prevotella* group	↑	Sobhani et al[39]
	CRC (*n*=46) HC (*n*=56)	454 pyrosequencing of the V3 region of the 16S ribosomal RNA gene	*Enterococcus*, *Escherichia/Shigella*, *Porphyromonas*, *Streptococcus*, *Peptostreptococcus*, and *Bacteroides fragilis*	↑	Wang et al[40]
			Bacteroides vulgatus, *Bacteroides uniformis*, *Roseburia*, *Alistipes*, *Eubacterium* and *Parasutterella*	↓	
	CRC (*n*=21) HC (*n*=22)	Pyrosequencing based analysis of the V1-V3 regions of the 16S rRNA genes	*Peptostreptococcus*, *Mogibacterium*, *Anaerococcus*, *Slakia*, *Paraprevotella*, *Anaerotruncus*, *Collinsella*, *Desulfovibrio*, *Eubacterium*, and *Porphyromonas*	↑	Chen et al[41]
	CRC (n=47) HC (n=94)	454 pyrosequencing of the V3 and V4 regions of the 16S ribosomal RNA gene; quantitative polymerase chain reaction	*Atopobium Fusobacterium*, and *Porphyromonas*	↑	Ahn et al[42]
			Ruminococcus	↓	
	CRC (n=19) HC (n=20)	Pyrosequencing of the V3 region of the 16S ribosomal RNA gene	*Fusobacterium/Bacteroides*	↑	Wu et al[43]
			Faecalibacterium prauznitsii/Roseburia	↓	
	CRC (*n*=10) HC (*n*=11)	Pyrosequencing of the V4 region of the bacterial 16S rRNA gene	*Acidaminobacter unclassified*, *Phascolarctobacterium unclassified*, *Citrobacter farmer*, *Akkermansia muciniphila*	↑	Weir et al[44]
			Bacteroides finegoldii, *Bacteroides intestinalis*, *Prevotella copri*, *Prevotella oris*, *Ruminococcus obeum*, *Dorea formicigenerans*, *Lachnobacterium bovis*, *Lachnospira pectinoschiza*, *Pseudobutyrivibrio ruminis*, *Bacteroides capillosus*, *Ruminococcus albus*, *Dialister invisus*, *Dialister pneumosintes*, *Megamonas hypermegale*	↓	
	CRC (n=53) HC (n=61)	Whole-genome shotgun sequencing	*Fusobacterium*, *Pseudoflavonifractor*, *Peptostreptococcus*, *Leptotrichia*, *Porphyromonas*, *Desulfovibrio*, *Parvimonas*, *Selenomonas*, *Bilophila*	↑	Zeller et al[45]
			Eubacterium, *Ruminococcus*, *Bifidobacterium*, *Campylobacter*, *Acinetobacter*	↓	
	CRC (n=46) HC (n=63)	Pyrosequencing	*Bacteroides*, *Fusobacterium*, *Alistipes*, *Escherichia*, *Parvimonas*, and *Bilophila*	↑	Feng et al[46]
			Ruminococcus, *Bifidobacterium*, and *Streptococcus*	↓	
	CRC (n=7) HC (n=10)	Real-time reverse transcription-PCR (qRT-PCR)	*Fusobacterium nucleatum*	↑	Fukugaiti et al[47]
	CRC (n=42) HC (n=89)	454 pyrosequencing of the V3 and V4 regions of the 16S ribosomal RNA gene	*Fusobacterium*, *Porphyromonas*	↑	Sinha et al[48]
			Clostridia, *Lachnospiraceae*	↓	

Continued

TABLE 1 (CONTINUED). Human clinical trials investigating faecal, cancer tissue and mucosa-adherent microbiota in CRC patients.

Sample type	Patient groups	Method	Outcome: variation in CRC compared to HC		Reference
	CRC (n=59) HC (n=49)	Terminal restriction fragment length polymorphism (T-RFLP) and next-generation sequencing (NGS) of the V3 and V4 regions of 16S rDNA	*Fusobacteria, Actinomyces, Atopobium, Haemophilus* genera; *Actinomyces odontolyticus*, *Bacteroides fragilis*, *Clostridium nexile*, *Fusobacterium varium*, *Heamophilus parainfluenzae*, *Prevotella stercorea*, *Streptococcus gordonii*, and *Veillonella dispar* species	↑	Kasai et al[50]
			Slackia genus; *Eubacterium coprostanoligensI* species	↓	
	CRC (n=74) HC (n=54)	Metagenomic sequencing; quantitative PCR (qPCR)	*Parvimonas micra, Solobacterium moorei, F. nucleatum, B. fragilis*	↑	Yu et al[51]
			Eubacterium ventriosum	↓	
	CRC (n=104) HC (n=102)	Quantitative real-time PCR (qPCR)	*F. nucleatum, Peptostreptococcus anaerobius and Parvimonas micra*	↑	Wong et al[52]
	CRC (n=203) HC (n=236)	Quantitative PCR (qPCR)	*Fusobacterium nucleatum, Clostridium hathewayi*	↑	Liang et al[53]
			Bacteroides clarus, Roseburia intestinalis	↓	
	CRC (n=50) HC (n=50)	Sequencing of the V3 and V4 regions of the 16S ribosomal RNA gene	*Escherichia-Shigella, Parvimonas, Fusobacterium, Porphyromonas*	↑	Yang et al[54]
			Firmicutes, Clostridiales, Clostridia, Lachnospiraceae, Ruminococcaceae, Selenomonadales, Negativicutes, and Faecalibacterium	↓	
Cancerous tissue samples					
	CRC (*n*=22) HC (*n*=22)	Real-time qPCR	*Bacteroides* species	↑	Sobhani et al[39]
	CRC (*n*=27)	pyrosequencing based analysis of the V1-V3 regions of the 16S rRNA genes	*Bacteroides, Prevotella,* and *Streptococcus*	↑	Chen et al[41]
			Lactobacillus, Roseburia, and *Pseudobutyrivibrio*	↓	
	CRC (*n*=11) HC (*n*=11)	RNA-seq; quantitative PCR	*F. nucleatum*	↑	Castellarin et al[55]
	CRC (*n*=95)	454 pyrosequencing of the V3 to V5 variable regions of the 16S rRNA genes; Quantitative real-time PCR	*Fusobacterium nucleatum, Streptococcaceae,*	↑	Kostic et al[56]
			Firmicutes and Bacteroidetes (Clostridia)	↓	
	CRC (n=48) HC (n=67)	454 pyrosequencing of the V1 and V3 regions of the 16S ribosomal RNA gene; qPCR	*Fusobacterium*	↑	McCoy et al[57]
	CRC (n=1102)	Quantitative PCR assay	*F. nucleatum*	↑	Mima et al[58]
	CRC (n=101)	Fluorescent quantitative polymerase chain reaction (FQ-PCR)	*F. nucleatum*	↑	Li et al[59]
	CRC (n=97) HC (n=6)	Real-time PCR	*Fusobacterium spp., E.faecalis, Bacteroides fragilis*	↑	Zhou et al[60]
	CRC (n=21) HC (n=56)	Sequencing of the V3 and V4 regions of the 16S ribosomal RNA gene	*Bacteroidetes Cluster 2, Firmicutes Cluster 2, Pathogen Cluster and Prevotella Cluster*	↑	Flemer et al[61]
			Bacteroidetes Cluster 1 and Firmicutes Cluster	↓	

Continued

TABLE 1 (CONTINUED). Human clinical trials investigating faecal, cancer tissue and mucosa-adherent microbiota in CRC patients.

Sample type	Patient groups	Method	Outcome: variation in CRC compared to HC		Reference
Mucosa-adherent microbiota					
	CRC (*n*=32) HC (*n*=22)	Pyrosequencing based analysis of the V1-V3 regions of the 16S rRNA genes	*Fusobacterium, Porphyromonas, Peptostreptococcaceae, Gemella, Mogibacterium,* and *Klebsiella*	↑	Chen et al[41]
			Faecalibacterium, Blautia, Anaeroslipes, Lachospira, and *Bifidobacterium*	↓	
	CRC (*n*=99) HC (*n*=61)	16S rRNA gene sequencing	*Fusobacterium, Gemella, Leptotrichia, B. fragilis, Peptostreptococcus, Parvimonas,*	↑	Nakatsu et al[62]
			Bacteroides and Blautia, F. prausnitzii, Sutterella, Collinsella aerofaciens, Alistipes putredinis	↓	

CRC: colorectal cancer; HC: healthy control.

Even though it is unknown if gut dysbiosis is a cause or a consequence of CRC, to explain the microbiota-related mechanism of carcinogenesis in colorectal cancer, scientists had proposed four hypotheses: the alpha-bug, the driver-passenger, the biofilm, and the bystander effect. The first one postulates that specific pathogenic bacteria, such as those previously mentioned, are able to induce colorectal cancer by producing toxins or by accelerating carcinogenic-related signalling. Differently, the driver-passenger hypothesis is founded on the assumption that some bacteria, defined passenger, are able to proliferate in the tumour environment, generated by the driver bacteria, leading to carcinogenesis. The biofilm hypothesis states the existence of a correlation between colorectal carcinogenesis and biofilm produced by gut microbiota, which involves the lack of E-cadherin or the activation of signal transducers and activator of transcription (STAT)-3and. The metabolites produced by the gut microbiota are the cornerstone of the bystander hypothesis. In this context, colorectal carcinogenesis may be related to the generation of CRC-promoting secondary bile acids; the metabolic activation or inactivation of pro-carcinogenic compounds, dietary phytochemicals, and xenobiotics; the hormone metabolism; the modification of inflammation pathways[36,37].

MICROBIAL METABOLIC PATHWAYS AFFECTING CARCINOGENESIS

Beyond gut microbiota dysbiosis and bacterial virulence factors, the microbial-derived metabolism is highly correlated with CRC development[63] since it is well known that microbial metabolites can exert genotoxic or tumor-suppressive functions[64]. In particular, both CRC initiation and progression of CRC could be related to changes in the metabolomic profiles, which in turn could be related to the alterations in the normal bacterial ecology[65]. In this context, the conversion of primary bile acids into secondary bile acids, by microbial derived metabolism, is suspected to be involved in colorectal carcinogenesis process, through apoptosis, cell proliferation, and DNA damage induction[22]. Some studies reported an increase of bacteria with β-glucuronidase activity in CRC patients[66], which play a central role in the metabolism of xenobiotics, suggesting their involvement in the initiation and progression of CRC[64,66]. In addition, products of protein fermentation, such as sulfides, ammonia, and nitrosamines, are classified as potentially toxic and pro-carcinogenic with proved involvement in CRC[64]. Sulfides, produced in the gut by bacterial reduction of dietary sulphate and other compounds[67], are enterotoxic[68] and have genotoxic effects on human cell lines at physiological concentrations[69]. As reported in Table 2, several studies aimed to characterize the metabolome of tissue and faecal samples collected from both CRC and healthy patients revealing changes in amino acid, glucose, lipid, and short chain fatty acids (SCFAs). In particular, an increase in amino acids and lactate, along with the alteration of intermediates of purines, pyrimidines, and the tricarboxylic acid (TCA) cycle were observed in tumour tissues[70-77]. Fumarate, as TCA intermediate[78], as well as glucose showed a decreasing trend in tissue profiling[79]. Differently, lactate, which derives from anaerobic glycolysis[80], was found at higher concentration in CRC tissues than in normal ones[79]. In addition, short-chain fatty acids (SCFAs) seem to be altered in CRC patients[79]. Notably, SCFAs are health-promoting bioactive molecules with anti-inflammatory properties and abilities to regulate the intestinal mucosal cell surface immune functions[81]. Evidence

TABLE 2. Human clinical trials investigating the metabolome of cancer tissue and faecal samples of CRC patients.

Sample type	Patients	Method	Outcome: variation in CRC compared to HC or adjacent mucosa		References
22 CRC tissues 25 Normal tissues	CRC (*n*=29)	High-resolution magic angle spinning nuclear magnetic resonance (HR-MAS NMR)	Cholinethreonine-containing compounds (ChoCC), taurine, scyllo-inositol, lactate and phosphocholine (PC)	↑	Chan et al[70]
			Lipids, polyethylene glycol (PEG) and glucose	↓	
31 CRC tissues 32 normal tissues	CRC (*n*=31)	Gas chromatography mass spectrometry (GC/MS)	Lactate, Phosphate, l-Glycine, 2-Hydroxy-3-methylvalerate, L-Proline, L-Phenylalanine, Palmitic acid, Marganic acid, Oleic acid, Stearic acid, Uridine, 11,14-Eicosadienoic acid, 11-Eicosenoic acid, 1-*O*-Heptadecylglycerol, 1-Monooleoylglycerol, Propyl octadecanoate, Cholesterol	↑	
			Fumarate, malate, mannose, galactose, glucose, 1-hexadecanol and arachidonic	↓	
12 CRC tissues 12 normal tissues	CRC (*n*=6)	Gas chromatography (GC/MS)	Lactate, L-Glycine, Palmitic acid, Marganic acid, Stearic acid, 1-O-Heptadecylglycerol, Propyl octadecanoate	↑	Mal et al[71]
			Malate, Creatinine enol, D-Mannose, D-Galactose, D-Glucose	↓	
16 CRC tissues 16 normal tissues	CRC (*n*=16)	Capillary electrophoresis time of flight mass spectrometer (CE-TOF/MS)	Lactate, succinate, and malate	↑	Hirayama et al[72]
			Glucose, pyruvate, acetyl CoA, citrate, *cis*-aconitate, iso-citrate, fumarate, and 2-oxoglutarate	↓	
12 CRC tissues 12 normal tissues	CRC (*n*=12) HC (n=12)	Two-dimensional NMR spectroscopy	Taurine, glutamate, choline	↑	Chae et al [73]
			Glucose, malate, and glycerol	↓	
127 CRC tissues 43 normal tissues	CRC (*n*=127) HC (*n*=43)	^{1}H nuclear magnetic resonance (^{1}H NMR)	Lactate, threonine, acetate, glutathione, sarcosine, uracil, succinate, serine, formate, lysine, tyrosine, leucine, valine, glutamine, alanine, serine, isoleucine	↑	Wang et al[74]
			Glucose, myo-inositol, taurine, phosphocreatine, creatine, betaine and dimethylglycine	↓	
Faeces	CRC (*n*=10) HC (*n*=11)	Gas chromatography (GC/MS)	Acetic acid, valeric acid, isobutyic acid, isovaleric acid	↑	Weir et al[44]
			Butyric acid	↓	
193 CRC tissues 163 normal tissues	CRC (*n*=193)	Gas chromatography–time-of-flight mass spectrometry (GC–TOFMS)	Kynurenine, β-Alanine, Glutamate, Cysteine, 2-Aminobutyrate, Palmitoleate, 5-Oxoproline, Aspartate, Hypoxanthine, Lactate, Myristate, Glycerol, Uracil, Putrescine, Hypotaurine, Spermidine, Homocysteine, 4-Aminobutyrate, Asparagine, Nicotinamide, AMP, Ascorbate, Glycine, Glyceraldehyde, Ornithine, Phosphate, Laurate, Galactose, 3-Methy-3-hydrpoxybutyrate, Methioninamide, 2-Aminoadipate	↑	Qiu et al[75]
			Myo-inositol, Glycerate, Glucose, Xylose	↓	
44 CRC tissues 44 normal tissues	CRC (*n*=44)	High Resolution Magic Angle Spinning (HR-MAS) NMR spectroscopy	Taurine, lactate, iso-glutamine, glycine, scyllo-inositol, glycerophosphorylcholine	↑	Mirnezami et al[76]

Continued

TABLE 2 (CONTINUED). Human clinical trials investigating the metabolome of cancer tissue and faecal samples of CRC patients.

Sample type	Patients	Method	Outcome: variation in CRC compared to HC or adjacent mucosa		References
Faeces	CRC (*n*=48) HC (n=102)	High-performance liquid phase chromatography and gas chromatography coupled with tandem mass spectrometry (HPLC-GC/MS-MS)	Heme, X_18565, X_19549, *p*-Hydroxybenzaldehyde, Mandelate, Palmitoyl-sphingomyelin	↑	Goedert et al[90]
			α-Tocopherol, γ-Tocopherol , Pterin, 4-Acetamidophenol, 2-Hydroxyacetaminophen sulfate, 3-Cystein-*S*-YL-acetaminophen, *p*-Acetamidophenylglucuronide, PABA, *N*-2-Furoyl-glycine, Sitostanol, Conjugated linoleate-18-2N7, 3-Dehydrocarnitine,	↓	
Faeces	CRC (n=42) HC (n=89)	High-performance liquid phase chromatography and gas chromatography coupled with tandem mass spectrometry (HPLC-GC/MS-MS)	p-hydroxy-benzaldehyde, palmitoyl-sphingomyelin	↑	Sinha et al[48]
			p-aminobenzoate, conjugated linoleate	↓	
Faeces	CRC (*n*=68) HC (n=32)	H nuclear magnetic resonance (H NMR)	Isoleucine, Leucine, Proline, Alanine, Valine, Glutamate, Dimethylglycine, Lactate, Succinate	↑	Lin et al[65]
			Glutamic acid, Glutamine, Valine, β-Glucose, Acetate, Butyrate, Propionate	↓	
17 CRC tissues 17 normal tissues	CRC (*n*=17)	Gas chromatography-mass spectrometry (GC/MS) ultra-performance liquid chromatography-mass spectrometry (UPLC-MS/MS)	Isobar: betaine aldehyde, N-methyldiethanolamine, adenylosuccinate, Isovalerate, Valerate, N1-methyl 2-pyridone-5-carboxamide	↑	Brown et al[91]
			2-aminoadipate, Stearoyl sphingomyelin, 4-hydroxyphenylpyruvate, Sorbitol, Alpha-hydroxyisovalerate, Cys-gly, oxidized, Tryptophylglycine, Deoxycholate, 7-ketodeoxycholate, Asparagine, Aspartylvaline, Aspartyltryptophan, Glucose-6-phosphate and fructose-6-phosphate	↓	
50 CRC tissues 50 normal tissues	CRC (*n*=50)	High-resolution magic-angle spinning (HRMAS) H NMR spectroscopy gas chromatography-flame ionization detector-mass spectrometer (GC-FID/MS)	Alanine, Aspartate, Choline, Cysteine, Cytosine, Glutamate, Glutamine, Glutathione, Glycerophosphocholine, Glycine, Isocytosine, Isoleucine, Lactate, Leucine, Phenylalanine, Phosphoethanolamine, Phosphorycholine, Sarcosine, *Scyllo*-inositol, Taurine, Tyrosine, Uracil, Valine	↑	Tian et al[77]
			Lipid	↓	
Faeces	CRC (n=50) HC (n=50)	gas chromatography-mass spectrometry (GC/MS)	Cadaverine, L-Proline, 1,4-Butanediamine, Urea, L-Glutamic acid	↑	Yang et al[54]
			Fructose, iditol, sedoheptulose, maltose, glycerol, galactosamine, 9, 12-octadecanoic acid, oleic acid, hexanedioic acid, and pentanedioic acid	↓	

CRC: colorectal cancer; HC: healthy control.

suggested that SCFAs are able to lower the intestinal pH, to act as energy sources for colonocytes, to stimulate the blood flow at colonic level, to secrete trans-epithelial chloride, and to stimulate the colonic epithelial cells proliferation[82]. In addition, SCFAs could stimulate the apoptosis cascade and regulate the histone hyperacetylation thus reducing the risk of cancer[83]. Compared to healthy control, altered levels of acetate, butyrate, propionate, and succinate were observed in CRC patients. Lin and co-workers[65] suggested that acetate and succinate could be considered as biomarkers in the early stage of CRC. In particular, the authors highlighted, at all stages of CRC, a downregulation of acetate, butyrate, and propionate whereas succinate was upregulated[65]. It is well known that acetate and butyrate provide energy to the intestinal cell wall[84] and their downregulation, due to the alteration of both intestinal and tissue microbiota, might be correlated to colorectal tumorigenesis[65]. Generally, butyrate, which is con-

sidered a microbial metabolite with anti-tumorigenic effects, seems to be able to reduce proliferation and to induce apoptosis in human colon carcinomas[85]. In addition, butyrate is associated with the decrease of colonic inflammation, the strength of the colonic barrier and the reduction of oxidative stress[86]. Even if several studies highlighted a positive role of butyrate in cancer prevention, its role in CRC remains debated and cannot be considered conclusive. In fact, some authors consider the available evidence as inconclusive due to discordances between *in vitro* and *in vivo* results[87,88] whereas others consider the potential anti-cancer effect of butyrate as unmistakable[89]. Overall, based on the aforementioned results, multiple dysregulated metabolites and in turn differences in metabolic pathways between CRC and healthy samples were highlighted. Nevertheless, there is no consensus about biomarker groups for CRC. For this reason, larger studies, addressing diverse populations, need to be designed and implemented.

PROBIOTICS IN CRC PREVENTION AND TREATMENT

Probiotic's ability to modulate gut microbiota in CRC patients and to prevent post-operative complications

Based on the central role played by gut microbiota in CRC promotion and progression, its modulation by probiotic administration could represent a valuable CRC-prevention strategy. In recent years, dietary strategies, including the administration of probiotics and prebiotics, were applied to modulate the composition and the metabolic activities of the intestinal microbiota. Probiotics, recognized as live bacteria which when administered in an adequate amount confer health benefits to the host[92], are able to exert health-promoting properties. Although strain-specific, these properties include the neutralization of cancerogenic compounds; the competition with pathogenic bacteria; the reconstruction of intestinal mucosal barrier and functionality by increasing the production of mucin, defensins, and immunoglobulin A (IgA) and by altering the pro-inflammatory cytokine and chemokine's response; the modulation and enhancement of the host's innate and adaptive immune response through the secretion of anti-inflammatory molecules and the regulation of helper T-cell. In addition, probiotics are able to increase the production of cytokines (IL-2 and IL-12), antioxidants, and anti-angiogenic factors; regulate apoptosis and cell differentiation; synthesize vitamins and short-chain fatty acids (SCFAs), nutrients, and growth signals for the intestinal epithelium; inhibit the tyrosine kinase signalling pathways. Pre- and probiotics, increasing at gut level the bioactive food components and microbial metabolites, could be useful to promote anti-tumour effect[13]. Several *in vitro* and *in vivo* studies, conducted on human cancer cell lines and on animal models, investigated the effects and the potential mechanisms exert by different probiotic strains in cancer inhibition. The emerging findings, which were extensively reviewed[93-95], suggest that probiotics exert intraluminal and systemic colorectal cancer-preventative effects. The main mechanisms involved are: competitively exclusion of pathogens[98,99], induction of change in intestinal microbiota enzymatic activity[100], reduction of carcinogenic secondary bile acids[101], binding of carcinogens and mutagens, increase SCFAs production, decrease DNA damage[102] and improvement of intestinal barrier function[103]. In addition, human clinical trials revealed that probiotics have inhibitory effect on the development of cancerous and precancerous lesions even though the effective mechanism is not fully understood. Table 3 summarizes the available clinical trials aimed to evaluate the effect of probiotics administration in CRC patients. Overall, results revealed that probiotics are able to modulate the gut microbiota composition in terms of dysbiosis normalization, to improve the intestinal barrier integrity, to inhibit the growth of pathogens, and to reduce the metabolism of pro-carcinogenic substances. In particular, probiotic administration to CRC patients can quantitatively and qualitatively modulate the gut microbiota composition enhancing both the abundance and the diversity of the microbiota to approach a balanced composition[104]. In a 12-week randomized, double-blind, placebo-controlled trial, CRC and polypectomized patients were treated with a symbiotic combination of oligofructose-enriched inulin, *Lactobacillus rhamnosus* GG and *Bifidobacterium lactis* Bb12 strains[105]. The improvement of epithelial barrier function, the reduction of both colorectal proliferation and capacity of faecal water to induce necrosis in colonic cells were observed. In addition, the treatment was able to induce significant changes in faecal microbiota with the increase of *Bifidobacterium* and *Lactobacillus* and the decrease of *Clostridium perfringens.* As demonstrated by Gianotti and co-workers[106], the pre and postoperative administration of a mixture of *Lactobacilli johnsonii La1* and *Bifidobacterium longum BB536* strains affected the intestinal microbiota composition by reducing the concentration of pathogens and by modulating the intestinal immune response. These effects were attributed to *L. johnsonii La1* strain based on its ability to adhere to the colonic mucosa and to colonize stool samples. The effect of pre and post-operative probiotic ad-

TABLE 3. Effects of pre and postoperative probiotic administration in CRC patients.

Probiotic strains	Patient groups	Study design	Dose and treatment	Outcome in patients subjected to probiotic administration	Reference
L. rhamnosus GG *Bifidobacterium lactis* Bb12 Inulin	CRC (*n*=37) Polypectomized (n=43)	RP-CT	10^{10} CFU orally administrated for 12 weeks	*Bifidobacterium* and *Lactobacillus* increased while *Clostridium perfringens* decreased in faeces; Reduced colorectal proliferation and the capacity of faecal water to induce necrosis in colonic cells; Improvement of epithelial barrier function and increase secretion of interleukin 2 by peripheral blood mononuclear cells in polypectomized patients; Incensement of interferon γ production in the cancer patients.	Rafter et al[105]
Lactobacillus johnsonii La1 Bifidobacterium longum BB536	CRC (n=31)	RP-CT	2×10^{7} CFU/day or 2×10^{9} CFU/day orally administrated	Reduction of the concentration of pathogens and modulation of the local immunity.	Gianotti et al[106]
Lactobacillus plantarum CGMCC 1258 *Lactobacillus acidophilus* LA-11 *Bifidobacterium longum* BL-88	CRC (n=100)	RP-CT	$2{,}6\text{x}10^{14}$CFU orally administrated for 6 days preoperatively and 10 days post-operatively	Increase of both diversity and microbial richness; *Bifidobacteria and Lactobacilli* increased while *Enterobacteriaceae, Pseudomonas and Candida* decreased.	Liu et al[107]
Bifidobacterium longum Lactobacillus acidophilus Enterococcus faecalis (1:1:1)	CRC (*n*=37) HC (*n*=11)	RP-CT	$6{,}0\times10^{7}$ CFU orally administrated for 5 days	Reduction in *Peptostreptococcus*, *Comamonas*, *Fusobacterium* and expansion of *Enterococcus* and Proteobacteria in the mucosa-adherent microbiota.	Gao et al[108]
Lactobacillus acidophilus LA-5 *Lactobacillus plantarum Bifidobacterium lactis* BB-12 *Saccharomyces boulardii* (LactoLevure®)	CRC (n=164)	RP-CT	10^{7} CFU one day before operation and continuing for another 15 days postoperatively	Reduction of the rate of all postoperative major complication (postoperative pneumonia, surgical site infections, anastomotic leakage. Shortened time until hospital discharge. Gene expression of *SOCS3* was positively related with gene expression of *TNF* and of circulating IL-6.	Kotzampassi et al[110]
Enterococcus faecalis T110 Clostridium butyricum TO-A Bacillus mesentericus TO-A (BIO-THREE®)	CRC (n=156)	RP-CT	six tablets orally daily administated	Enhancement of the immune responses and improvement of the intestinal microbial environment by determining the increase of bifidobateria; Reduction of superficial incisional surgical site infections in patients undergoing CRC surgery.	Aisu et al[111]
Bifidobacterium longum Lactobacillus acidophilus Enterococcus faecalis (Bifico)	CRC (n=60)	RP-CT	≥ $1{,}0\text{x}10^{7}$ CFU 5 days before and 7 days after CRC resection operation	Faster recovery of bowel function, lower incidences of diarrhea, and lower rate of bacteraemia.	Yang et al[112]
Lactobacillus acidophilus NCFM *Bifidobacterium animalis* subsp. *lactis* Bl-04	CRC (n=15) HC (n=15)	RP-CT	$1{,}4\times10^{10}$ CFU *B. lactis* Bl-04 and 7×10^{9} CFU *L. acidophilus* NCF M for 31±28	Modulation of the microbiota composition, enrichment of butyrate-producing bacteria	Hibberd et al[109]
Lactobacillus acidophilus NCFM *Bifidobacterium animalis* subsp. *lactis* Bl-04	CRC (n=15) HC (n=15)	RP-CT	$1{,}4\times10^{10}$ CFU *B. lactis* Bl-04 and 7×10^{9} CFU *L. acidophilus* NCF M for 31±28 days; range 8–78 days	Modulation of the microbiota composition, enrichment of butyrate-producing bacteria	Hibberd et al[109]

Continued

TABLE 3. Effects of pre and postoperative probiotic administration in CRC patients.

Probiotic strains	Patient groups	Study design	Dose and treatment	Outcome in patients subjected to probiotic administration	Reference
Lactobacillus acidophilus NCFM *Lactobacillus rhamnosus* HN001 *Lactobacillus paracasei* LPC-37 *Bifidobacterium lactis* HN019 and fructo-oligosaccharides	CRC (n=91)	RP-CT	10^9 CFU twice a day for 5 days before the surgical procedure and for 14 days after surgery.	Reduction of postoperative infection rates	Flesh et al[113]

CRC: colorectal cancer; HC: healthy control; RP-CT: Randomized placebo-controlled trial.

ministration was also evaluated by Liu and co-workers[107]. The study showed that the administration of *Lactobacillus plantarum* CGMCC 1258, *Lactobacillus acidophilus* LA-11n and *Bifidobacterium longum* BL-88 (2.6x10^{14}CFU) for 6 days preoperatively and 10 days post-operatively determined the increase of the gut microbiota diversity and richness in CRC subjects undergoing a colorectomy. At the end of the treatment, the intestinal microbiota composition of patients resembled that of the healthy individuals[107]. This result agrees with the evidence that emerged in a prospective randomized controlled trial conducted by Gao and co-workers[108]. *Bifidobacterium longum, L. acidophilus* and *Enterococcus faecalis* administration for 5 days was able to counteract the low diversity of the gut microbiota of CRC patients and to effectively reduce pathogenic *Fusobacterium* and *Peptostreptococcus* populations. Similarly, Hibberd and colleagues[109] investigated the intestinal tissue and faecal samples microbiota of CRC patients, that received or did not receive probiotics, and of healthy controls. The MiSeq analysis of the V4 variable region of the 16S rRNA gene of bacteria and archaea revealed, after cluster analysis, a significant shift in the microbiota composition. In fact, the microbiota profile of mucosa and tumour samples collected from treated CRC was significantly different compared to CRC placebo patients and healthy control[109]. Overall, CRC patients that received probiotics had a unique microbiota profile characterised by an increased abundance of butyrate-producing bacteria in tumour, mucosa, and faecal samples compared with patients with cancer who did not receive probiotics. In particular, *Clostridiales* spp. and *Faecalibacterium* were enriched in both tissue and faecal samples obtained from CRC patients subjected to probiotic administration. *Eubacterium* was elevated in faecal and mucosa samples whereas *Roseburia* and *Lachnospira* were higher in mucosa and tumour samples from patients that received the probiotic. The CRC-associated taxa, *Fusobacterium* and *Peptostreptococcus*, were less abundant in faecal samples of patients that received the probiotics[109].

Human clinical trials also showed that probiotics administration might be a promising approach to prevent post-operative complications in patients undergoing abdominal surgery. Some of these recent findings are summarized in Table 3. A double-blind, placebo-controlled randomized study evaluated the ability of *Lactobacillus acidophilus* LA-5, *Lactobacillus plantarum, Bifidobacterium lactis* BB-12 and *Saccharomyces boulardii* probiotic stains to reduce post-operative complications on CRC patients undergoing colorectal surgery[110]. In particular, a significant decrease in the rate of postoperative major complications, such as postoperative pneumonia, surgical site infections, and anastomotic leakage was observed in patients subjected to probiotics time administration. The hospital discharge was shortened and the gene expression of *SOCS3* was positively related to gene expression of *TNF* and of circulating IL-6 in the probiotic group but not in the placebo group[110]. Similar results were achieved by Aisu and co-workers[111] in CRC patients subjected to *Enterococcus faecalis T110 Clostridium butyricum TO-A Bacillus mesentericus TO-A* probiotic strains administration in patients undergoing colorectal cancer surgery. Compared to the placebo group, the probiotic one showed a significant reduction of surgical site infection incidence and an increase in $CD4^+$ATP activity along with an increase in the ratio of beneficial bacteria in faeces. The anti-infective effects of perioperative treatment with *Bifidobacterium longum, L. acidophilus,* and *Enterococcus faecalis* probiotic strains in patients receiving confined CRC respective surgery was studied[112]. Overall, the days to the first flatus and the days to the first defecation were significantly improved in patients treated with probiotics. In addition, the incidence of diarrhea was significantly lower in the probiotic group than in the control one. Therefore, perioperative probiotic administration significantly influenced the recovery of bowel function, which may reduce the short-term infectious complications such

as bacteremia[112]. Recently, the perioperative use of symbiotic (*Lactobacillus acidophilus* NCFM, *Lactobacillus rhamnosus* HN001, *Lactobacillus paracasei* LPC-37, *Bifidobacterium lactis* HN019 and fructo-oligosaccharides) significantly reduced the incidence of wound infection and remote infections such as pneumonia[113].

Based on these evidences, further studies should be conducted in a larger population. To better understand the role of probiotics in CRC prevention and treatment microbiota data should be complemented with metabolomics information. In addition, the potential influences of fungi (mycobiome) and viruses (virome) should be investigated.

Probiotics to manage cancer treatment side effects

Probiotics are very attractive as a potential adjuvant therapy in preventing and/or reducing GI side effects due to anticancer treatment improving the compliance of patients. In fact, probiotics administration could help in re-establish both the abundance and the functionality of the commensal gut bacteria, which could have been depleted after the therapies[114]. In spite of the probiotic administration to immunocompromised cancer patients could theoretically represent a risk of opportunistic infections and of potential transfer of antibiotics resistance[115], their use in several trials has shown encouraging results related to the re-establishment of healthy intestinal microbiota composition, the amelioration of diarrhoea and other types of therapy-associated side-effects[116]. The effectiveness of probiotic administration in mitigating the adverse gastrointestinal effects of cancer treatment was firstly demonstrated in animal models. Interestingly, Bowen and collaborators[117], using a mouse experimental model, highlighted the ability of the VSL#3 probiotic treatment to reduce the severity of diarrhea and to improve histological examination. The anti-diarrhoeic effect of probiotic administration (*Lactobacillus casei* variety *rhamnosus* Lcr35 or *L. acidophilus* and *Bifidobacterium bifidum* strains) was also revealed by Yeung and co-workers[118], using mice subjected to 5-Fluorouracil (5-FU) intraperitoneally injection. Recently, using a CRC rat model, it was possible to demonstrate that the *Bifidobacterium infantis* administration resulted in a considerable attenuation of chemotherapy-induced intestinal mucositis. In addition, a decrease in the level of proinflammatory cytokines (IL-6, IL-1β, TNF-α) and an increase of CD4+ CD25+ Foxp3+ T regulatory cell response was observed[119]. According to that, several clinical studies have investigated the therapeutic potential of the gut microbiota manipulation in cancer patients through oral administration of probiotics, along with anticancer treatment. Strains belonging to *Lactobacillus* and *Bifidobacterium* species along with *Enterococcus faecalis, Saccharomyces boulardii*, *Streptococcus thermopilus,* and *Leuconostoc mesenteroides* strains have been extensively studied[120] confirming their usefulness in the improvement of diarrhea and intestinal peristalsis; reduction of enterocolitis; modulation of gut microbiota composition, regulation of intestinal immune functions; decrease serum zonulin and septicaemia[120]. An investigation study[121], conducted on 150 CRC patients, randomly allocated to receive *Lactobacillus rhamnosus* GG (LGG) and fibre or placebo, showed that patients treated with LGG had significantly less severe grades of diarrhoea and less abdominal discomfort, thereby reducing the need for hospital care and lowering of chemotherapy doses. As shown by a randomised controlled trial, the administration of *L. acidophilus* and *B. bifidum* prevent intestinal toxicity in CRC patients treated with both radiotherapy and cisplatin[122]. Similarly, the oral administration of a mix of 10 bacterial strains (including *Lactobacilli* and *Bifidobacteria*) during irinotecan-based chemotherapy resulted in an effective reduction of diarrhoea and gastrointestinal dysfunctions[123]. A decreased risk of developing post-operatory irritable bowel syndrome (IBS) was found in CRC patients subjected to resection when co-treated with a symbiotic mix of prebiotics and probiotics[124]. Interestingly, the perioperative probiotic administration was proved to be advantageous in reducing post-operative infection rates[113]. In addition to the aforementioned studies, several clinical trials are ongoing with the aim to evaluate safety and efficacy of the probiotics administration during anticancer therapy[125]. Based on the aforementioned scientific data is evident that not all the probiotics are useful or carry out the same action so variations of probiotic strains, doses, and regimens are needed to obtain the desired effect. Although positive feedback clearly emerged, more in-depth information are needed to give a consensus about the use of probiotics as adjunctive therapy for a better outcome against the detrimental effects of anticancer therapies.

FUTURE PERSPECTIVES AND PROMISING FIELD

Overall, even if the correct cascade of events leading intestinal dysbiosis, inflammation and CRC risks is not completely clear, it seems reasonable the assumption that the re-establishment of the gut microbiota balance represents a key element to support the host's anti-cancer defence and to reduce the therapy-related toxicity. Microbiota transplantation,

including faecal microbiota transplantation (FMT) and selective microbiota transplantation (SMT), may improve the effectiveness of anti-cancer treatment and/or reduce the related side effects. Even if the microbiota transplantation presents some limitations related to methodology, potential adverse events, insufficient clinical evidence and ethical issues, its application in oncology seems to be promising. In particular, in experimental animal models, FMT and SMT seem to be effective before anti-cancer treatment in reconstitute gut microbiota and improve the immune status of the host as well as in the enhancement of the effectiveness of oncotherapy reducing tumour resistance and adverse events[126,127]. Clinical studies and case reports demonstrated the benefit of faecal microbiota transplantation in *Clostridium difficile* infection (CDI) in cancer patients. In fact, CDI is the most common cause of antibiotic-associated diarrhoea, leading to high morbidity and mortality in cancer patients. Hefaziet and co-workers[128] studied the effectiveness of FMT in 23 cancer patients with recurrent CDI subjected to cancer chemotherapeutic agents. Interestingly, the effective rate was 86% without serious adverse reactions or infectious complications. No infectious complications resulted from FMT even in immunocompromised patients who under-went FMT[129]. In addition, FMT was successfully applied to treat severe CDI refractory to conventional antibiotics treatment in hematopoietic stem cell transplantation patients[129-131]. Based on the aforementioned evidence, FMT is promising in alleviating different cancers linked to intestinal dysbiosis and cancer treatment-associated complications. Additionally, FMT could enhance the efficacy of cancer immunotherapy, thus remarkably affect clinical trials outcomes. However, large-sample randomized controlled are required to delineate the validity of FMT, especially focus on the long-term consequences.

More interestingly, the use of complementary and alternative medicine (CAM), which comprises a wide range of products, such as herbs, vitamins, minerals, probiotics, and medical practices, such as acupuncture or magneto-therapy, is growing in oncologic patients[132-137]. However, few scientific papers, especially in Europe, have evaluated CAM in cancer patients[138].

CONCLUSIONS

Scientific evidence has demonstrated that gut microbiota plays a central role in patients' responses to anticancer therapies as well as in clinical efficacy and sensitivity to toxic side effects. The intestinal microbiota characterization has strongly improved knowledge about its composition and the change occurring in CRC. Nevertheless, more in-depth studies, involving metabolomics and metatranscriptomics approaches, should be carried out in order to better understand the interactions between host and pathogens correlated with colorectal carcinogenesis. Although traditional cancer therapies are still the mainstream treatments, probiotics have gained increasing attention based on the preventive action against the onset and for the treatment of CRC. In fact, probiotics seem to be capable of significantly ameliorate the patients' compliance to treatments as well as their overall quality of life. Despite the already published *in vivo* results, demonstrating the beneficial effect of probiotics in alleviating the side-effects of anticancer therapies, to fully understand their action further randomized double-blind, placebo-controlled clinical trials are strongly required to recommend their routine use as adjunctive therapy for CRC prevention and treatment. In addition, a personalized approach, which takes into account the subject-specific clinical and pathological background, should be adopted in order to gain only the positive outcomes of probiotics administration, avoiding harmful side-effects.

Conflict of Interest:

The authors declare no conflict of interest.

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