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REVIEW

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Microbial metabolites in colorectal tumorigenesis and cancer therapy

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ABSTRACT

Trillions of microbes are indigenous to the human gastrointestinal tract, together forming an ecological community known as the gut microbiota. The gut microbiota is involved in dietary digestion to produce various metabolites. In healthy condition, microbial metabolites have unneglectable roles in regulating host physiology and intestinal homeostasis. However, increasing studies have reported the correlation between metabolites and the development of colorectal cancer (CRC), with the identification of oncometabolites. Meanwhile, metabolites can also influence the efficacy of cancer treatments. In this review, metabolites derived from microbes-mediated metabolism of dietary carbohydrates, proteins, and cholesterol, are introduced. The roles of pro-tumorigenic (secondary bile acids and polyamines) and anti-tumorigenic (short-chain fatty acids and indole derivatives) metabolites in CRC development are then discussed. The impacts of metabolites on chemotherapy and immunotherapy are further elucidated. Collectively, given the importance of microbial metabolites in CRC, therapeutic approaches that target metabolites may be promising to improve patient outcome.

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Introduction

The human gastrointestinal tract harbors trillions of microbes to form an ecological community known as the gut microbiota. There are approximately 3.8×10^{13} bacteria colonized in the gut with more than 1,500 species predominantly belonging to phyla Bacteroidetes and Firmicutes, followed by Proteobacteria, Fusobacterium, Actinobacteria, and Verrucomicrobiota, together accounting for 90% of total microbes in the gut microbiota.[1,2] To date, it is widely accepted that the gut microbiota profoundly affects human physiology and health.[3] With the advancement of microbial profiling technology particularly metagenomic sequencing, the understanding toward the gut microbiota has become clearer and more comprehensive.[4] Gut commensal microbes play important roles in maintaining intestinal physiology and homeostasis, including producing antimicrobial substances to protect the host from pathogen infection, regulating the host immune system, facilitating the digestion process, and mediating the integrity of intestinal barrier.[3,5,6] However, the gut microbiota is readily influenced by a variety of extrinsic factors, including diet, age, and use of antibiotics. These environmental factors can cause imbalanced composition and altered function of the gut microbiota, leading to microbial dysbiosis, a pathogenic process that has been associated with numerous diseases such as inflammatory bowel disease, metabolic disorder, neurodegenerative disease, and cancer.[7–9]

During metabolism of dietary components, gut commensal microbes produce various intermediate, or end products known as metabolites involving short-chain fatty acids (SCFAs) and indole derivatives. These microbes-derived metabolites signal through their homologous receptors on host cells to contribute the maintenance of normal physiology[10]. Of note, accumulated evidence has indicated that gut metabolites are closely engaged with different diseases including colorectal cancer (CRC). In particular, a dysbiotic microbiota could produce harmful metabolites to interfere with the host immune system, leading to the release of genotoxic virulence factors, and eventually promoting

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colorectal tumorigenesis.[11,12] For example, elevated level of secondary bile acids (SBAs) especially deoxycholic acid (DCA), has been implicated in the development of CRC.[13] In contrast to these oncometabolites, recent findings have identified microbial metabolites that are able promote cancer treatment efficacy.[14,15] In this review, we summarize gut metabolites produced from microbes-mediated metabolism of dietary components including carbohydrates, proteins, and cholesterol. The roles of tumor-promoting metabolites such as SBAs and polyamines as well as tumor-suppressing metabolites including SCFAs and indole derivatives in CRC development are then explored. We further discuss how microbial metabolites influence the efficacy of chemotherapy and immunotherapy, and highlight that understanding the relationship between metabolites and cancer can assist the development of novel therapeutic strategy targeting metabolites to improve clinical outcome.

Microbial metabolism in healthy gut

The gut microbiota is closely associated with the digestion and absorption of dietary components. During metabolism, various metabolites are produced by gut microbes which further interact with host cells for physiological processes and functions.[16,17] In this section, products of microbes-mediated metabolism of dietary carbohydrates, proteins, and cholesterol are explored.

Products of carbohydrate fermentation

Certain types of dietary carbohydrates, including resistant starch, polysaccharides from plant cell wall, and indigestible oligosaccharides, cannot be digested by host enzymes.[18] Instead, these carbohydrates are metabolized by gut bacteria, and SCFAs are produced after fermentation. SCFAs are saturated aliphatic organic acids consisting of up to 6 carbons, with acetate, propionate, and butyrate being the most common SCFAs which account for over 95% of total SCFAs.[19] The breakdown of carbohydrates to SCFA involves a variety of bacterial enzymes. Indigestible carbohydrates are first hydrolyzed by microbial enzymes (e.g., polysaccharidases and glycosidases) to five- or six-carbon monosaccharides, which are further catabolized as a fermentation substrate to produce SCFAs.[20]

Through metagenomic sequencing, gut bacteria responsible for SCFA production have been characterized (Table 1). Acetate producers are widely distributed among the gut microbiota which include *Prevotella spp., Ruminococcus spp., Bifidobacterium spp., Bacteroides spp., Clostridium spp., Streptococcus spp., A. muciniphila, and B. hydrogenotrophica*.[21] In comparison, propionate production is more conserved and only a few bacteria are involved. Bacteroidetes and Negativicutes were reported as the dominant bacterial taxa responsible for the conversion from succinate to propionate,[22] while Bacteroidetes abundance was found to be positively correlated with propionate level in human fecal samples.[23] On the other hand, since enzymes involved in butyrate synthesis (e.g., butyryl-CoA dehydrogenase, butyryl-CoA transferase, butyrate kinase) are commonly expressed in the gut microbiota, there are many gut bacteria capable of producing butyrate, including *Ruminococcus bromii*, *Faecalibacterium prausnitzii, Eubacterium rectale, Eubacterium hallii, Anaerostipes hadrus*, and *Coprococcus catus*. Of note, although many bacteria are butyrate producers, they share different substrates during their fermentation of dietary carbohydrates. For instance, *Ruminococcus spp. and E. rectale* are able to ferment diet-derived polysaccharides such as starch, arabinoxylan and inulin, compared to *F. prausnitzii* which has limited ability to degrade dietary polysaccharides.[24,25]

Products of protein metabolism

Most dietary proteins are first degraded into small fragments by proteases in gastric juice. After these protein fragments reach the intestines, they are hydrolyzed into amino acids by host endopeptidases and microbial proteases.[26] These amino acids are further fermented by various microbes-mediated metabolism, producing polyamines, phenols, and indole derivatives. Polyamines such as putrescine, spermidine, and spermine, are small polycationic molecules produced from microbes-mediated fermentation of arginine, and this process requires the enzyme amino acid decarboxylase. *Bacteroides spp*. and *Fusobacterium spp*. are the

Table 1. SCFA-producing bacteria in human gut.

SCFAs	Bacteria	Fecal relative abundance (%)	Substrate	Bacterial genes for carbohydrate degradation
Propionate	*Bacteroides fragilis*	3.70556	succinate	phosphoenolpyruvate carboxykinase
	Bacteroides uniformis	7.09498	succinate	methylmalonyl-CoA decarboxylase
	Bacteroides vulgatus	9.10405	succinate	methylmalonyl-CoA decarboxylase
	Bacteroides thetaiotaomicron	2.38777	succinate	methylmalonyl-CoA decarboxylase
	Selenomonas ruminantium	0.04098	succinate	methylmalonyl-CoA decarboxylase
	Veillonella parvula	6.05763	succinate	methylmalonyl-CoA decarboxylase
	Propionibacterium freudenreichii	0.0231	succinate	succinate decarboxylase
	Propionibacterium acidipropionici	0.01208	succinate	succinate decarboxylase
	Prevotella copri	18.25712	succinate	unknown
	Alistipes putredinis	6.94845	succinate	unknown
	Dialister invisus	2.86624	succinate	methylmalonyl-CoA decarboxylase
	Dialister succinatiphilus	2.07663	succinate	methylmalonyl-CoA decarboxylase
	Phascolarctobacterium succinatutens	5.75006	succinate	methylmalonyl-CoA decarboxylase
	Blautia obeum	0.40752	succinate	propanediol dehydratase
	Bifidobacterium adolescentis DSM 20,083	0.79354	succinate	methylmalonyl-CoA decarboxylase
	Roseburia inulinivorans	0.59039	succinate	CoA-dependent propionaldehyde dehydrogenase
	Megasphaera elsdenii	0.60836	lactose	lactoyl-CoA dehydratase
	Clostridium botulinum	0.05975	lactose	lactoyl-CoA dehydratase
	Clostridium novyi	0.00719	lactose	lactoyl-CoA dehydratase
Butyrate	*Faecalibacterium prausnitzi*	4.06159	acetyl-CoA	butyryl-CoA:acetate CoA-transferase
	Roseburia hominis	0.27651	acetyl-CoA	butyryl-CoA:acetate CoA-transferase
	Roseburia intestinalis	0.6059	acetyl-CoA	butyryl-CoA:acetate CoA-transferase
	Roseburia inulinivorans	0.59039	acetyl-CoA	butyryl-CoA:acetate CoA-transferase
	Eubacterium hallii	0.044	acetyl-CoA	butyryl-CoA:acetate CoA-transferase
	Eubacterium limosum	0.06078	acetyl-CoA	butyryl-CoA:acetate CoA-transferase
	Eubacterium ramulus	0.08001	acetyl-CoA	butyryl-CoA:acetate CoA-transferase
	Eubacterium ruminantium	0.11911	acetyl-CoA	butyryl-CoA:acetate CoA-transferase
	Eubacterium cellulosolvens	0.065	acetyl-CoA	butyryl-CoA:acetate CoA-transferase
	Eubacterium hallii	0.313	acetyl-CoA	butyryl-CoA:acetate CoA-transferase
	Clostridium perfringens	5.49137	acetyl-CoA	butyryl-CoA:acetate CoA-transferase
	Clostridium butyricum	7.41686	acetyl-CoA	butyrate kinase
	Anaerostipes hadrus	0.0577	acetyl-CoA	butyryl-CoA:acetate CoA-transferase
	Clostridium kluyveri	0.00203	4-aminobutyrate	butyryl-CoA:4-hydroxybutyrate CoA transferase
	Anaerostipes butyraticus	0.0576	acetyl-CoA	butyryl-CoA:acetate CoA-transferase
	Anaerostipes caccae	0.3643	4-aminobutyrate	butyryl-CoA:4-hydroxybutyrate CoA transferase
	Clostridium saccarobutylicum	0.08999	glutarate	butyrate kinase
	Ruminococcaceae bacterium	0.09346	glutarate	butyryl-CoA:acetate CoA-transferase
	Coprococcus catus	0.0812	acetyl-CoA	butyrate kinase
	Coprococcus comes	0.33764	acetyl-CoA	butyrate kinase
	Coprococcus eutactus	0.94995	acetyl-CoA	butyrate kinase
	Butyrivibrio crossotus	3.90884	acetyl-CoA	butyrate kinase
	Butyrivibrio fibrisolvens	0.01713	acetyl-CoA	butyryl-CoA:acetate CoA-transferase
	Butyrivibrio proteoclasticus	0.01315	acetyl-CoA	butyrate kinase
	Shuttleworthia satelles	0.03472	acetyl-CoA	butyrate kinase
	Subdoligranulum variabile	0.00335	acetyl-CoA	butyryl-CoA:acetate CoA-transferase
	Anaerococcus hydrogenalis	1.9246	acetyl-CoA	butyrate kinase
	Anaerococcus lactolyticus	0.0928	acetyl-CoA	butyrate kinase
	Anaerococcus prevotii	0.70814	acetyl-CoA	butyrate kinase
	Anaerotruncus colihominis	0.23757	acetyl-CoA	butyryl-CoA:acetate CoA-transferase
	Megasphaera micronuciformis	0.10935	acetyl-CoA	butyryl-CoA:acetate CoA-transferase
	Halanaerobium praevalens	0.00059	acetyl-CoA	butyryl-CoA:acetate CoA-transferase
	Porphyromonas asaccharolytica	0.05103	lysine	butyryl-CoA:acetoacetate CoA transferase
	Porphyromonas endodontalis	0.0106	lysine	butyryl-CoA:acetoacetate CoA transferase
	Porphyromonas gingivalis	0.0049	lysine	butyryl-CoA:acetoacetate CoA transferase
	Alkaliphilus metalliredigens	0.01554	lysine	butyryl-CoA:acetoacetate CoA transferase
	Alkaliphilus oremlandii	0.00629	lysine	butyryl-CoA:acetoacetate CoA transferase
	Fusobacterium gonidiaformans	0.09302	lysine	butyryl-CoA:acetoacetate CoA transferase
	Fusobacterium nucleatum	3.72779	lysine	butyryl-CoA:acetoacetate CoA transferase
	Carboxydibrachium pacificum	unknown	lysine	butyryl-CoA:acetoacetate CoA transferase
	Carboxydibrachium hydrogenoformans	unknown	lysine	butyryl-CoA:acetoacetate CoA transferase
Acetic acid	*Bifidobacterium longum*	0.3797	acetyl-CoA	acetic kinase
	Bifidobacterium animalis	0.05631	acetyl-CoA	acetic kinase
	Faecalimonas umbilicata JCM 30,896	unknown	formate	formate acetyltransferase
	Parabacteroides	5.72002	unknown	unknown
	Clostridium acetobutylicum	0.48542	acetyl-CoA	acetic kinase
	Desulfovibrio vulgaris	0.02778	unknown	unknown
	Akkermansia muciniphila	2.20562	unknown	unknown
	Bacteroides thetaiotaomicron	2.38777	acetyl-CoA	acetate kinase

main producer of polyamines in the gut.[27] While *Enterococcus faecalis* together with *Escherichia coli* can induce putrescine production, of which arginine is converted to agmatine by arginine decarboxylases in *E. coli*, then putrescine is synthesized from agmatine by sequential reactions catalyzed by *E. faecalis*.[28] Moreover, several species from *Bacteroides* and *Parabacteroides* have carboxyspermidine decarboxylase, a crucial enzyme for the production of spermidine.[29]

Phenols are the major end product from bacterial fermentation of aromatic amino acid tyrosine. Multiple gut bacterial taxa including Fusobacteriaceae, Enterobacteriaceae, Coriobacteriaceae, and *Clostridium* clusters I and XIVa were reported to harbor homologs of tyrosine lyase or hydroxyphenylacetate decarboxylase which are involved in the final steps of phenol production[30]. Similarly, indole and indole derivatives are produced from bacteria-mediated tryptophan metabolism by the action of species-specific enzymes. Currently, over 85 bacterial species such as *E. coli*, *Clostridium spp*., and *Bacteroides spp*., were identified to express enzymes capable of catalyzing the direct conversion from tryptophan to indole. Of note, bacterial tryptophan-related enzymes such as aromatic amino acid aminotransferase and decarboxylase, indole acetamide dehydrogenase, indoleamine 2,3-dioxygenase, and indolelactic acid dehydratase, are highly varied among species, hence different bacteria with distinct enzymes cooperate with each other to facilitate host tryptophan metabolism.[31] For example, *Clostridium sporogenes* and *Clostridium bartlettii* express tryptophan aminotransferase to facilitate tryptophan conversion to indole-3-pyruvic acid.[32] Whereas in *Burkholderia pyrrocinia*, tryptophan is degraded to indole-3-acetamide by tryptophan 2-monooxygenase, following by conversion to indole-3-acetic acid by indole acetamide dehydrogenase expressed in several *Lactobacillus* and *Bacteroides* spp.[33]

Products of cholesterol metabolism

Primary bile acids are produced from cholesterol oxidation in the liver, followed by conjugation with either taurine or glycine, and being transported to the intestines. Notably, intestinal conjugated bile acids need to be unconjugated in order to facilitate lipid metabolism, and this process is closely mediated by bacteria. Gut bacteria expressing bile salt hydrolase are responsible for hydrolyzing conjugated bile acids into unconjugated SBAs. Bile salt hydrolase is mostly expressed in gram-positive bacteria including *Clostridium, Enterococcus, Bifidobacterium*, and *Lactobacillus*. Whilst other bacterial enzymes such as 7α/β-hydroxysteroid dehydrogenase are also important for bile acid maintenance. For example, 7a/β-hydroxysteroid dehydrogenase mediates SBA production by removing the 7a/β-hydroxy group from primary bile acids, and this enzyme was reported to be predominantly expressed in *Clostridium spp*., namely *C. scindens, C. hiranonis*, and *C. hylemonae*. Moreover, 7α/β-hydroxysteroid dehydrogenases in several *Clostridium spp*. especially *C. absonum and C. baratii* could facilitate the production of secondary ursodeoxycholic acid, which is further dehydroxylated by *Eubacterium spp*. to form lithocholic acid, one of the most toxic SBAs produced in the intestines.[34,35]

Microbial metabolites in CRC

Changes of metabolite profile in CRC

With the advent of high-throughput metabolomic analysis, the link between microbial metabolites and CRC has become clearer (Figure 1). The most commonly used methods for metabolomic profiling include mass spectrometry coupled with different separation techniques (e.g., gas chromatography, liquid chromatograph, capillary electrophoresis, matrix-assisted laser desorption/ionization) and nuclear magnetic resonance. Numerous studies have depicted the gradual changes of metabolites in serum, fecal, and mucosal samples of patients with CRC, compared to healthy individuals. Notably, despite the inter-cohort variation, several meta-analyzes have shown microbial metabolic pathways that are consistently changed across different studies. In 2019, a meta-analysis of 768 fecal samples revealed an increased amino acid degradation in CRC patients.[36] Consistently, multiple studies identified the significant alteration of amino acids in the

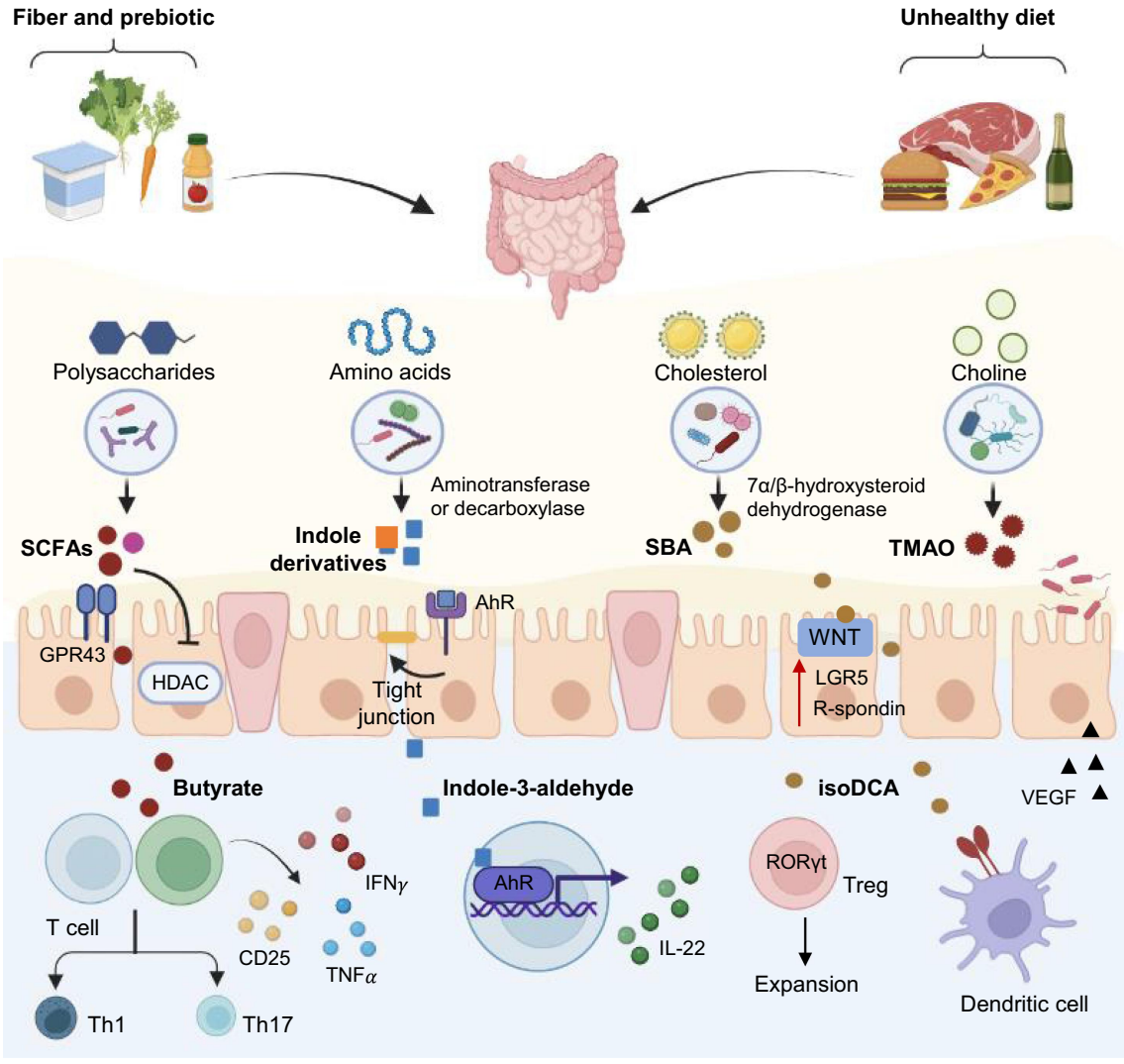

Figure 1. Gut microbial metabolites in CRC development. Different metabolites are produced by microbes-mediated metabolism of various dietary components. Some microbial metabolites including SCFAs and indole derivatives are protective against colorectal tumorigenesis by regulating epigenetic modification, maintaining intestinal barrier integrity, and modulating host immunity. While other metabolites such as SBA and TMAO contribute to CRC development by activating the oncogenic WNT pathway and promoting an immunosuppressive microenvironment that favors the growth and survival of tumor cells. GPR43, G protein-coupled receptor 43; RORγt, retinoid orphan receptor γt; VEGF, vascular endothelial growth factor.

progression of CRC[37]. Of which, alanine, tyrosine, asparagine, aspartic acid, tryptophan, methionine, and phenylalanine were found to be decreased significantly in CRC patients,[36–38–40] whereas glutamic acid, glycine, histidine, and isoleucine were upregulated.[41] In another meta-analysis of 624 samples, Thomas *et al.* identified the overexpression of choline trimethylamine-lyase genes in CRC patients, suggesting a relationship between microbial choline metabolism and CRC[42]. Consistent with this finding, a metabolomic profiling study confirmed the upregulation of trimethylamine N-oxide (TMAO) in tumor tissues of patients with advanced CRC,[43] while free choline was also found to be downregulated in serum and feces of CRC patients from other studies.[44,45]

Apart from amino acids and their derivatives, alterations in lipids and lipid-related molecules were also frequently identified in patients with CRC. For instance, a study of plasma metabolite profile reported that palmitic acid and linoleic acid are significantly increased in patients with sporadic CRC, compared to healthy individuals.[46] Palmitic acid is a saturated fatty acid and its association with CRC tumorigenesis has been consistently reported.[47] Other lipid metabolites including

acetate, succinate, and lactate in fecal and tissue samples of patients with early CRC were also found to be significantly distinct from healthy individuals.[48,49] Collectively, these findings demonstrated the close correlation between changes of gut metabolites and CRC.

Metabolites function as CRC biomarkers

Given that changes in the gut metabolome are associated with CRC progression, these altered metabolites may have translational potential as biomarkers for CRC diagnosis. Indeed, through untargeted metabolomic profiling, a study in 2019 demonstrated that the enrichment of polyamines is correlated with CRC phenotype, and the significantly altered metabolites could discriminate CRC patients from healthy individuals.[50] The changes in gut metabolome can also be utilized to identify individuals with increased risk of CRC. For example, a study investigated the progression from precancerous adenomas to CRC based on integrated microbial and metabolomic profiling on fecal samples. The results showed that in addition to the enriched *Fusobacterium, Parvimonas*, and *Staphylococcus*, cholesteryl esters and sphingolipids also serve as biomarkers to discriminate CRC from patients with advanced adenomas.[51] Interestingly, combining metabolites biomarkers to the microbial fingerprint model could improve diagnostic accuracy of CRC with an area under the curve (AUC) of 0.928. Similarly, our recent integrated study identified a set of 20 fecal metabolites with significant correlation to CRC, of which norvaline and myristic acid are consistently increased along the adenoma-carcinoma sequence.[52] Moreover, combining 11 metabolites biomarkers with 6 bacterial species including *F. nucleatum*, *Peptostreptococcus anaerobius*, and *Parvimonas micra* could further improve the discriminating power in CRC diagnosis with an AUC of 0.94. Taken together, these studies suggested that combining metabolites and bacterial biomarkers may be promising for developing a better and more accurate CRC diagnosis. In addition, apart from fecal metabolites, an integrated study has identified a set of serum metabolites that are able to distinguish patients with CRC and adenoma from healthy individuals with an AUC of 0.98, which is even higher than the clinically used biomarker carcinoembryonic antigen.[53]

Microbial metabolites in tumorigenesis

Tumor-promoting metabolites

Secondary bile acids

Bile acids, especially SBAs, are widely considered as oncometabolites in the development of CRC, of which the aberrant accumulation of SBAs in CRC has been observed in many clinical studies (Table 2).[54–56] In general, increased intestinal SBAs concentration can reshape the composition of gut microbiota to induce microbial dysbiosis with enriched opportunistic pathobionts and depleted beneficial commensals, eventually contributing to CRC development. For instance, a preclinical study revealed that DCA treatment could decrease the abundances of *Lactobacillus gasseri* and multiple butyrate-producing bacteria such as *Clostridium leptum*, *Lachnospiraceae bacterium*, and *Eubacterium coprostanoligenes* in mice, accompanied by impaired intestinal barrier, low-grade inflammation, and accelerated tumor progression.[57] Of note, using antibiotics to deplete microbiota in mice markedly alleviated DCA-induced tumorigenesis, suggesting that DCA promotes CRC development through modulating microbiota composition. Moreover, as one of the most effective antimicrobial bile acids, DCA can significantly inhibit the growth of gut beneficial commensals including *Lactobacillus* and *Bifidobacterium*.[58]

SBAs are important signaling molecules that regulate both innate and adoptive immune responses. Numerous studies have demonstrated that SBAs can remodel host immunity to promote tumorigenesis. For example, isoalloLCA and 3β-hydroxydeoxycholic acid (isoDCA) are two SBAs that can promote the expansion of immunosuppressive regulatory T cells (T_{reg}), of which isoalloLCA and isoDCA respectively induce T_{reg} differentiation with increased FOXP3 expression in naïve $CD4^+$ T cells and farnesoid X receptor activity in dendritic cells.[68] Besides, supplementation of a bioengineered *Bacteroides* strain capable of producing isoDCA led to increased number of colonic RORγt-expressing T_{reg} in mice, indicating

Table 2. Amino acids and lipid metabolites associated with CRC.

Type	Metabolite	Trend	Sample type	Study type	Ref
Amino acid	Lysine	up	tissue, stool	prospective	43,50
	Leucine	up	tissue, stool	prospective	39
	Phenylalanine	up	tissue, stool, serum	pro/retrospective	52,59
	Serine	up	tissue	retrospective	59
	Isoleucine	up	tissue, stool	prospective	39,60
	Tryptophan	down	tissue	prospective	43
	Tyrosine	up	tissue, stool	prospective	50,60
	glutamine	up	tissue	prospective	61
	Glutathione	up	tissue	prospective	61
	Alanine	up	tissue, stool	prospective	52,60
	Taurine	up	tissue	prospective	61
	Glycine	up	stool	prospective	52
	Proline	up	stool	prospective	50
	Valine	up	stool	prospective	50
	Aspartate	up	serum	prospective	62
	Hypoxanthine	up	serum	prospective	62
	Indole-3-acetaldehyde	up	stool	prospective	52
Lipid	Sphingolipids	up	stool	prospective	63
	Lithocholic acid	up	stool, colon fluid	prospective	64
	Chenodeoxycholic acid	up	stool, colon fluid	prospective	64
	Deoxycholic acid	up	stool, colon fluid	prospective	64
	Cholic acid	up	stool	prospective	65
	Choline	up	stool	prospective	63
	Cholesterol	up	stool, serum	prospective	66,67

the direct inducing effect of isoDCA on T_{reg}.[69] Apart from T_{reg}, SBAs can also inhibit the infiltration of natural killer T (NKT) cells to dampen antitumor immune responses. In particular, SBAs promote liver tumor growth by inhibiting CXCL16-dependent accumulation of hepatic NKT cells, whereas depleting SBA-producing gram-positive bacteria by antibiotics was sufficient to rescue hepatic NKT cell accumulation and suppress tumor growth.[70]

In addition, SBAs also promote colorectal tumorigenesis by activating oncogenic pathways including TGR5/STAT3, WNT/β-catenin, and NF-kB signaling.[71–74] A recent study in 2022 reported that SBAs could enhance stemness of CRC stem cells, of which SBAs involving tauro-β-muricholic acid and DCA induce proliferation of Lgr5-expressing cancer stem cells, further driving malignant transformation and promoting the adenoma-carcinoma progression.[75] Consistently, oral administration of DCA to mice led to increased colonic expression of R-spondin 3, an important protein regulating stem cell fate, whereas depletion of R-spondin 3 prevented such DCA-induced stem cell proliferation. These findings thus suggest that DCA contributes to the formation of precancerous neoplasia through upregulating R-spondin 3 to drive expansion of intestinal stem cells.

Polyamines

The correlation between polyamines and CRC is well-established, of which most polyamines especially N1, N12-diacetylspermine are significantly increased in patients with CRC.[76] High intake of spermine is also associated with increased risk of CRC with an adjusted odd ratio of 1.58.[77] Polyamine upregulation in tumor tissues is mainly mediated by ornithine decarboxylase, the key rate-limiting enzyme for polyamine biosynthesis. As ornithine decarboxylase is a transcriptional target of the oncogenic MYC, MYC hyperactivation in tumors leads to upregulated polyamine biosynthesis.[78] Ornithine decarboxylase activity can also be activated by RAS, one of the most commonly mutated oncogenes, hence activated RAS in tumor cells could significantly increase polyamines uptake.[79] Given the crucial role of polyamines in tumor tissues, suppressing polyamine level combined with targeting oncogenes may be a potential therapeutic strategy against CRC.[80,81]

Polyamines exhibit pro-tumorigenic effects via interacting with the gut microbiota. An observational study reported a direct correlation between bacterial biofilm formation and the upregulation of N1, N12-diacetylspermine in CRC patients.[82] Mechanistically, bacterial polyamine metabolites could promote bacterial biofilm formation to

create a microenvironment favorable for oncogenic transformation in colonic epithelial cells. Another polyamine, spermidine, is synthesized by and required for colibactin-producing *E. coli* to exhibit genotoxic activity,[83] hence suggesting that spermidine could promote colorectal tumorigenesis through increasing the pathogenicity of colibactin-producing *E. coli*.

In addition, polyamines can act as immunomodulators to influence CRC development. Immunosuppressive cells including myeloid-derived suppressor cells, dendritic cells, and monocyte-derived M2 macrophages rely on polyamine metabolism to support their growth and function in suppressing the immune system. For example, polyamine biosynthesis induces a specific chemical modification (hypusination) of the enzyme eIF5A that regulates the activation signals of metabolic switching between oxidative phosphorylation and glycolysis in macrophages.[84] Moreover, another study reported that polyamines suppress lymphocyte proliferation, interleukin (IL)-2 production, macrophage-mediated tumoricidal activity, and neutrophil locomotion.[85] Taken together, polyamines can interact with both microbes and host immune cells to contribute colorectal tumorigenesis.

Trimethylamine N-oxide

Excess dietary intakes of red meat and fats are well-established risk factors of CRC. When the gut microbiota digests these food, TMAO is generated as metabolites which has been reported to be associated with CRC.[86] In a case-control study, plasma TMAO level was found to have positive correlation with the risk of CRC.[87] Similarly, another observational study showed that serum TMAO level is significantly increased in CRC patients when compared to healthy individuals, and patients with higher TMAO level have reduced survival rate, implicating the potential of serum TMAO as a prognostic marker of CRC.[88]

Although accumulated studies have demonstrated the correlation between TMAO and CRC, the direct evidence proving its role in CRC is lacking. A recent study in 2022 showed that TMAO could enhance the secretion of vascular endothelial growth factor A to promote CRC cell proliferation *in vitro*, whilst long-term choline feeding upregulates circulating TMAO level, causing formation of new blood vessels and increased tumor growth in mice.[89] TMAO can also interact with gut microbes to promote colorectal tumorigenesis. In the study by Yoo *et al.*, mice fed with long-term high-fat diet developed low-grade mucosal inflammation with increased *E. coli*-mediated choline catabolism, which further elevates circulating TMAO level.[90] Mechanistically, high-fat diet impairs mitochondrial bioenergetics in the colonic epithelium to increase the luminal bioavailability of oxygen and nitrate, thereby promoting *E. coli* growth and intensifying its mediated choline catabolism. Notably, drugs reducing oxygen availability could decrease TMAO level to restore normal intestinal physiology.

Other tumor-promoting metabolites

The advance of metabolomic profiling has facilitated the characterization of metabolites that are previously undescribed. For instance, Cao *et al.* discovered a family of novel metabolites termed as indolimines using integrated untargeted metabolomics and electrophoresis-based bioactivity-guided fractionation techniques.[91] These indolimines are produced by CRC-associated *Morganella morganii* and they exhibit genotoxic functions by causing DNA damage, subsequently promoting colorectal tumorigenesis in mice. Moreover, tumor cells in CRC are surrounded by various components including blood vessels, immune cells, and fibroblasts, together they form the tumor microenvironment (TME). TME is known to have a high concentration of wastes that are generated by metabolic reprogramming. Interestingly, a recent study identified a robust increase of ammonia in a mouse model of metastatic CRC.[92] Ammonia is a waste produced by gut microbes after the breakdown of host proteins by microbial ureases.[93] Due to its cytotoxicity, ammonia must be exported and processed by the liver through the urea cycle.[94] Meanwhile, the accumulation of ammonia in TME reduces the function of antitumor T cells and promotes T cell exhaustion, whereas improving ammonia clearance could reactivate T cells, decrease tumor growth, and extends survival of mice.[92] Altogether, these findings imply that harmful microbial metabolites are not only

capable of promoting colorectal tumorigenesis, but also impacts advanced CRC progression and metastasis.

Tumor-suppressing metabolites

Bacteriocin

Bacteriocins are peptides synthesized by bacterial ribosomes with bacteriostatic and/or bacteriolytic activity. Accumulated evidence has shown that bacteriocins could attenuate the growth of tumor cells via various mechanisms. For example, tumor cell membrane predominantly carries a negative charge with higher membrane fluidity and contains more microvilli as compared with non-tumor cells, whereas these characteristics allow selective binding of bacteriocins to tumor cells. Such interaction could increase membrane fluidity and form ion channels on tumor cell membranes, thereby increasing the accumulation of intracellular reactive oxygen species and promoting tumor cell apoptosis and necrosis. In addition to inducing cytotoxicity, bacteriocins can suppress tumor growth by exhibiting anti-inflammatory and immunomodulatory effects. These include modulating cytokines secreted by colonic epithelial cells, downregulating pro-inflammatory pathways, promoting the secretion of antimicrobial agents from epithelial cells to kill pro-inflammatory bacteria, and strengthening the intestinal barrier to reduce invasion of pro-inflammatory pathogens. Bacteriocins can also enhance phagocytosis of macrophages, collectively boosting the antitumor immunity in the immunosuppressive TME.[95,96]

Among all characterized bacteriocins, bacteriocins produced by lactic acid bacteria especially nisin are the most studied bacteriocins. Nisin is secreted by *Lactococcus lactis* with a wide range of tumor-suppressing effects. Multiple *in vitro* studies reported that nisin could increase apoptosis of CRC cells by altering gene expressions (BAX and BCL-2),[97] and suppress proliferation, migration, and invasion of CRC cells via downregulating associated genes (CEA, CEAM6, MMP2F, MMP9F).[98] Nisin also exhibited immunomodulatory activity by inhibiting pro-inflammatory cytokines IL-1β and IL-6 production and enhancing the percentage of $CD4^+$ $CD8^+$ T cells in innate immune cell population.[99]

Plantaricin is another type of bacteriocins produced by lactic acid bacteria possessing antitumor activity. In particular, laterosporulin10 from *Brevibacillus laterosporus* SKDU10, pediocin CP2 from *Pediococcus acidilactici* MTCC 5101, and microcin E492 from *Klebsiella pneumoniae* were all reported to be capable of inducing tumor cell apoptosis through targeting cell membranes directly.[100–102] Plantaricin EF from *Lactobacillus plantarum* was reported to increase tight junction protein ZO-1 and alleviate the effect of pro-inflammatory cytokines (IL-8) on disrupted intestinal epithelial barrier in mice with diet-induced obesity.[103] Meanwhile, microcin could promote the growth of probiotic *E. coli* Nissle 1917 meanwhile limiting the expansion of competing Enterobacteriaceae and pathogenic *Salmonella enterica* to alleviate intestinal inflammation.[104] Similarly, the administration of microcin J25 to mice with enterotoxigenic *E. coli* infection led to reduced intestinal colonization of pathogens, improved intestinal morphology, and decreased intestinal permeability and inflammatory pathology.[105]

Short-chain fatty acids

SCFAs especially acetate, propionate, and butyrate are metabolites well-known for their benefits against CRC. A recent integrated metagenomic and metabolomic analysis revealed the decline in butyrate-producing bacteria coupled with acetate reduction in CRC patients, indicating that fecal butyrate level may be a potential biomarker of CRC risk or represent an early warning signal of the disease onset, progression and severity.[60] In general, SCFAs suppress colorectal tumorigenesis via immunomodulatory activity, whereas free fatty acid receptor 2 (FFAR2, alternatively known as GPR43), a major receptor of SCFAs, is necessary for SCFAs to exhibit anti-CRC effects. In a transgenic mouse model of CRC, loss of FFAR2 led to increased tumor growth and impaired intestinal barrier with higher frequencies of exhausted T cells and overactivated IL-27-expressing dendritic cells, thus suggesting that SCFAs are essential for the maintenance of immune response against colorectal tumors.[106] Indeed, SCFAs were reported

to influence T cell differentiation into helper T cells (Th)-1, Th17, and other effector T cells through their inhibitory activity on histone deacetylase.[107] Particularly, pentanoate and butyrate could enhance the activity of cytotoxic $CD8^+$ T cells through metabolic and epigenetic reprogramming, resulting in an elevated production of antitumor molecules such as CD25, interferon (IFN)-γ and tumor necrosis factor (TNF)-α[14]. Recent study also highlighted the role of butyrate in promoting the generation of memory T cells through reprogramming cellular mitochondrial metabolic flux.[108] Apart from enhancing the antitumor functions of effector T cells, butyrate could modulate T_{reg} expansion and the stimulation of conventional T cells in a dose-dependent manner. Specifically, low concentration of butyrate facilitates T_{reg} differentiation via engagement with FFAR2 to exhibit anti-inflammatory effect,[109] whereas high concentration of butyrate could induce the expression of transcription factor T-bet and IFN-γ secretion from $CD4^+$ T cells through inhibiting the activity of histone deacetylase, thereby augmenting antitumor immunity[110].

In contrast to normal epithelial cells, tumor cells prefer to undergo glycolytic pathway rather than oxidative phosphorylation as their energy resource, and this feature is commonly known as Warburg effect. As a consequence, glucose instead of butyrate is more likely to be consumed by tumor cells, resulting in butyrate accumulation in nuclei of tumor cells. Of note, accumulated butyrate in the nucleus could inhibit the activity of histone deacetylase, limit proliferation and metastasis, and promote apoptosis of tumor cells.[111–113] Intracellular butyrate could also induce autophagy-mediated degradation of β-catenin to prevent its translocation to nucleus for undergoing oncogenic transcriptional activity, further suppressing tumor cell proliferation.[114] Apart from butyrate, a recent study in 2022 has revealed the antitumor effect of propionate through epigenetic modification, of which propionate is able to promote proteasomal degradation of EHMT2, leading to the reduction of H3K9me2 level on the promoter region of TNFAIP1, subsequently inducing apoptosis in tumor cells.[115] Given the widespread antitumor features of SCFAs, approaches to upregulate SCFAs such as enriching SCFA-producing bacteria may potentially facilitate the prevention and control of CRC. Indeed, the administration of butyrate-producing bacteria *Clostridium butyricum* significantly suppressed the oncogenic Wnt/β-catenin signaling pathway through activating FFAR2, leading to inhibition of tumor development in $Apc^{min/+}$ mice, a transgenic mouse model of CRC.[116]

Indole derivatives

Indole and its derivatives such as indole lactic acid and indole acetic acid, are the products of bacteria-mediated tryptophan metabolism and have been demonstrated to inhibit the development of CRC in multiple studies.[117,118] Indole derivatives are the main ligands of the aryl hydrocarbon receptor (AhR), and their interaction is fundamental for various physiological functions including the maintenance of intestinal immune homeostasis and protection against pathogen infection. In particular, indoleacrylic acid could activate AhR to increase mucin expression in epithelial cells for improving intestinal barrier, and upregulate anti-inflammatory IL-10 secretion from immune cells for mitigating inflammation.[119] Dietary tryptophan also increases colonization of *Lactobacillus* in the gut, and these colonized *Lactobacillus* then secrete indole-3-aldehyde to protect intestinal mucosa from inflammation and infection by pathogenic fungi via activating AhR-dependent IL-22 transcription.[120] Other indole derivatives including indole-3-ethanol, indole-3-pyruvate, and indole-3-aldehyde were also found to be capable of improving intestinal barrier function in an AhR-dependent manner.[121] Interestingly, emerging evidence has demonstrated the association of indole derivatives with intestinal stem cells. For example, a co-culture system with intestinal organoids and lamina propria lymphocytes indicated that indole-3-aldehyde first activates AhR to stimulates IL-22 secretion by lymphocytes, then promotes proliferation of $Lgr5^+$ stem cells via inducing STAT3 phosphorylation to accelerate recovery from intestinal damage.[122]

In terms of antitumor capability, a preclinical study reported that dietary supplementation of an AhR agonist, indole-3-carbinol, could significantly restore intestinal barrier homeostasis and protect the stem cell niche, thereby preventing colorectal

tumorigenesis.[123] In a recent study published in 2021, Sugimura *et al.* discovered a novel probiotic species *Lactobacillus gallinarum* that could directly exert anti-CRC effect.[118] Mechanistically, *L. gallinarum* secreted indole 3-lactic acid to induce apoptosis and suppress proliferation and growth of CRC cells, whilst indole 3-lactic acid supplementation alone was sufficient to reduce tumor load in transgenic $Apc^{min/+}$ mice. Moreover, blocking AhR using antagonist abolished the anti-CRC effect of *L. gallinarum*, suggesting that the tumor-suppressive activity of *L. gallinarum* is dependent on the interaction between indole 3-lactic acid and AhR.

Microbial metabolites in cancer treatment

Chemotherapy

Gut microbial metabolites are closely associated with chemotherapeutic pharmacokinetics, efficacy, and toxicity. In particular, some studies reported that metabolites can modulate chemotherapy efficacy by inducing an immunostimulatory TME that favors the drugs to exhibit their toxicity on tumor cells, in turn promoting drug-induced immunogenic cell death (Figure 2). For instance, oxaliplatin which is one of the first-line chemotherapeutic drugs of CRC, showed reduced antitumor efficacy in antibiotics-treated mice, whereas supplementation of butyrate could restore oxaliplatin-induced tumor regression.[124] Consistently, clinical data also supported that serum butyrate level is positively associated with oxaliplatin efficacy in cancer patients. In TME, butyrate strongly promotes IFN-γ production by $CD8^+$ T cells through epigenetic upregulation of pro-inflammatory IL-12 signaling, enhancing antitumor immunity and oxaliplatin efficacy. Similarly, another recent study revealed that metabolite peptidoglycan derived from *Bifidobacterium bifidum*, a probiotic species capable of boosting antitumor immune response, activates IFN-γ signaling to improve oxaliplatin efficacy.[124]

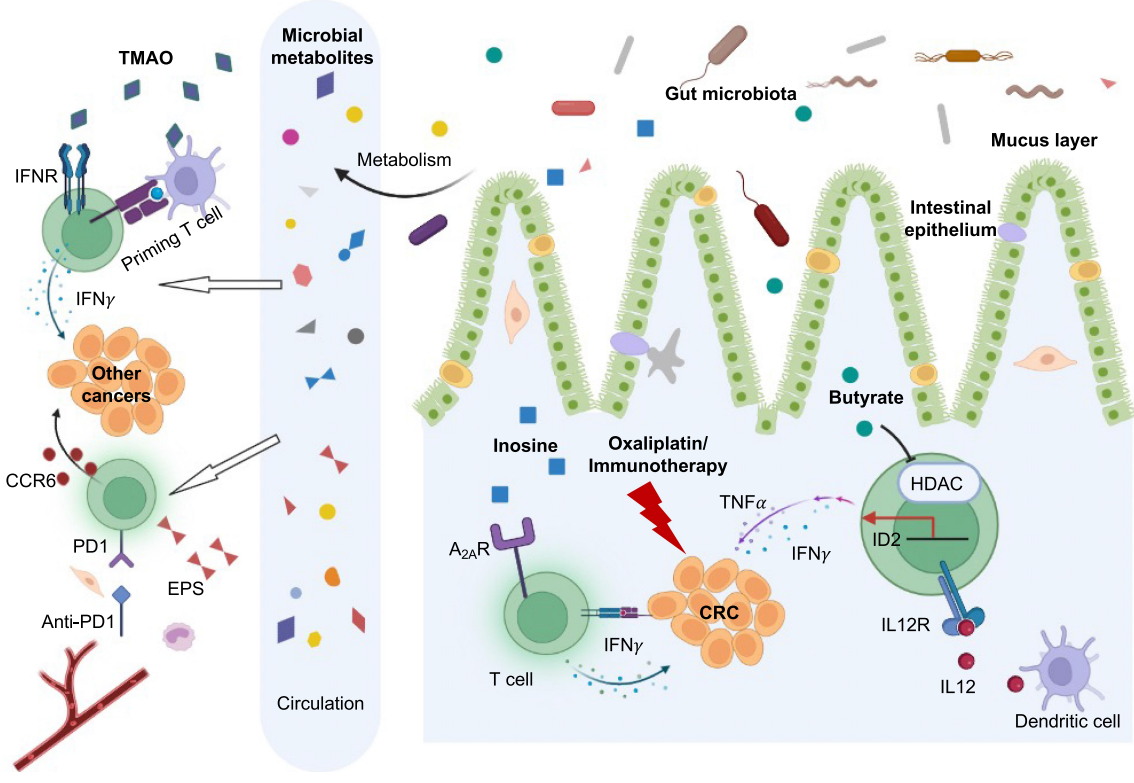

Figure 2. Gut microbial metabolites in cancer treatment. Microbial metabolites have varied effects on cancer treatments including chemotherapy and immunotherapy. Butyrate and inosine can improve treatment efficacy against CRC by boosting the antitumor immune responses by dendritic cells and effector T cells, respectively. Meanwhile, some metabolites such as TMAO and EPS can enter the circulation and reach tumor tissues that are outside from the gut, eventually influencing treatment efficacy against these cancers. EPS, exopolysaccharides; ID2, inhibitor of DNA binding 2; IFNr, interferon production regulator.

5-FU is another widely used chemotherapeutic drug for CRC. However, its efficacy is frequently limited in CRC patients of which 5-FU-resistant tumor cells can exhibit stem cell-like properties with self-renewal potential and increased invasion and metastasis. Interestingly, the culture supernatant of *L. plantarum* was reported to selectively inhibit the stem cell characteristics of 5-FU-resistant CRC cells *in vitro* by downregulating stemness markers (CD44, CD133, and ALDH1) and inactivating the WNT/β-catenin signaling.[125] In another study, Kim *et al.* identified that metabolites produced by *L. plantarum* have a synergistic effect with butyrate to exhibit tumor-suppressing activity through restoring the functional expression of SMCT1 (a major transporter of butyrate) in 5-FU-resistant CRC cells.[126] Urolithin A (3,8-dihydroxybenzo[c]chromen-6-one, UroA) is a microbial metabolite derived from dietary phenol metabolism and exerts anti-inflammatory, antioxidative, and anti-aging effects.[127,128] In a recent study, UroA or its analogue UAS03, was found to synergistically act with 5-FU to enhance treatment efficacy, as evidenced by increased apoptosis and decreased proliferation and invasiveness in 5-FU-resistant CRC cells.[129] Mechanistically, UroA and UAS03 regulate transcription factors (FOXO3, FOXM1) to reduce the downstream expression and activity of multidrug resistance-associated protein 2 (MRP2; a drug efflux transporter contributing to 5-FU resistance), resulting in effective 5-FU chemotherapy. Taken together, these studies illustrate the close association of metabolites with chemotherapy efficacy. Utilizing gut metabolites as chemotherapy adjuvants may be a novel and potential clinical strategy to overcome chemotherapeutic resistance in CRC.

Immunotherapy

Since the past decade, immunotherapy, especially immune checkpoint inhibitors (ICIs), has become a major treatment option for a variety of solid tumors, including a subset of CRC (MMR-D/MSI-H phenotype). ICIs aim to restore and enhance antitumor immune responses by inhibiting tumor-intrinsic immunosuppressive pathways. In general, ICIs work by using fully humanized monoclonal antibodies against the two most studied immune checkpoints – cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and programmed cell death protein 1 (PD-1) or its ligand PD-L1. Of note, the responsiveness of ICIs is highly varied among patients, ranging between 30% and 50% in patients with MMR-D/MSI-H CRC, not to mention that ICIs are not recommended in patients with other CRC subtypes due to poor efficacy.[130] Given that the crosstalk between microbial metabolites and immune cells plays an essential role in maintaining immune homeostasis, multiple studies have searched for metabolites that can affect immunotherapy efficacy. For instance, the recent study by Mager *et al.* discovered that the metabolite inosine produced by *Bifidobacterium pseudolongum* could significantly enhance ICI response with strengthened antitumor immunity in CRC mouse model.[131] Due to the immunotherapy-induced intestinal barrier disruption, inosine could be systemically transferred and subsequently activated the adenosine A_{2A} receptor on T cells, thereby regulating Th1 differentiation and increasing intratumoral infiltration of IFN-γ^+CD4$^+$ and CD8$^+$ T cells.

TMAO is derived from microbes-mediated metabolism of dietary choline and it can induce inflammation and immune activation.[132,133] In a mouse model with pancreatic ductal adenocarcinoma, TMAO administration decreased tumor burden through driving immune activation of dendritic cells and cytotoxic T cells, and such TMAO-induced immune-activated TME could contribute to improved ICI efficacy.[134] Mechanistically, TMAO first potentiates the type I IFN pathway in macrophages to promote their activation, while the activated macrophages then enhance effector T cell response, further sensitizing pancreatic tumors to ICIs. Similarly, another study of triple-negative breast cancer reported the reduced tumor growth after TMAO administration, of which TMAO could promote intratumoral infiltration of CD8$^+$ T cells and pro-inflammatory M1 macrophages to strengthen antitumor immunity.[135] Moreover, clinical data showed that patients with high plasma TMAO level achieve better response to ICIs, and the abundance of bacteria containing choline trimethylamine-lyase (an enzyme involved in TMAO production) such as *Bacillus* is significantly increased in ICI responders.[134,135] Collectively, these studies

implicated the therapeutic potential of TMAO administration against different cancers, which transiently transforms the immunosuppressive TME into an immunogenic state that can respond to ICI.[134] Notably, TMAO is widely considered as oncometabolites in CRC as aforementioned, thus these contrasting findings imply the difference between protumorigenic effect and therapeutic potential of TMAO. Special caution is therefore needed when utilizing TMAO as adjuvants of ICIs or other treatments against CRC, and extensive investigations are necessary prior to its clinical application.

Exopolysaccharides are extracellular metabolites secreted by microbes to their living environment. A recent study in 2022 demonstrated that exopolysaccharides produced by *Lactobacillus* are able to enhance ICI efficacy in tumor-bearing mice.[136] More specifically, these microbial exopolysaccharides bind to receptors on $CD8^+$ T cells (LPAR2) to induce CCR6 expression, augmenting the expression of IFN-γ and genes encoding IFNγ-inducible chemokines, thereby enhancing antitumor T cell function with improved ICI efficacy. In hence, given the inseparable connection between microbial metabolites and host immune cells, targeting the gut metabolome seems to be practical to improve immunotherapy responsiveness. Several strategies including the application of engineered microbes or specific diet to reshape the gut microbiota and produce target metabolites have been reported.[137,138] Ideally, with deeper understanding of the functions of microbial metabolites in TME, more novel approaches can be developed to enhance immunotherapy efficacy and eventually improve patient outcome.

Future perspective

The recent advance in high-throughput metabolomic profiling has brought major breakthrough in characterizing the gut metabolome in humans. In normal conditions, gut metabolites and microbes cooperate together to perform physiological functions and contribute to host health, while their abnormal alterations can lead to pathogenesis and tumorigenesis. Particularly, the enrichment of both oncometabolites and pathogenic microbes is closely associated with CRC development, while some probiotics or beneficial commensals protect against tumorigenesis by producing antitumor metabolites. Given by their importance, increasing research has tried to utilize metabolites in feces or serum/plasma metabolites as clinical biomarkers for CRC diagnosis and prognosis, whereas a combination of bacterial and metabolites biomarkers could achieve even better diagnostic performance.[52] Nevertheless, it is noteworthy that currently there are only a few metabolites with consistent enrichment or depletion among studies, probably due to the highly dynamic nature of gut metabolome in different human populations. Extensive work is therefore needed to identify a universal consortium of metabolites biomarkers that can be utilized as a standard diagnosis for CRC.

Although chemotherapy and immunotherapy are major treatments of CRC, their efficacy is varied among patients and enormous efforts have been invested to improve therapeutic responsiveness. The gut microbiota and metabolome are now well acknowledged for their impacts on cancer treatment. Approaches that modulate microbiota to improve treatment efficacy including antibiotics and fecal microbiota transplantation, have been heavily investigated, yet these strategies have different limitations. For example, increasing evidence has reported the negative correlation between antibiotics use and treatment outcome,[139–141] while fecal microbiota transplantation involves the transfer of unknown or uncharacterized microbes which may be harmful to the recipient. In comparison, metabolites are more specific and less influenced by inter-individual disparity once their biological functions are evaluated.[142–144] A metabolite can be either beneficial or harmful to humans based on its function, while such characterization is unsuitable for microbes as a species could be beneficial to an individual but potentially harmful to another person. In general, current findings on the therapeutic application of metabolites are mostly based on preclinical animal studies, and metabolites with immunomodulatory functions particularly SCFAs and indole derivatives exhibit robust results to improve treatment efficacy. Nonetheless, it remains unclear whether supplementing a single or cocktail of metabolites could improve patient outcome in clinical setting. Meanwhile, safety is another concern on the

use of metabolites specifically regarding TMAO. Although temporary TMAO administration was reported to be beneficial, its intrinsic pro-tumorigenic function should be aware, and investigations are needed to identify the safe range of treatment dosage and time for TMAO supplementation. Altogether, microbial metabolites have emerged as a robust alternative to microbes in clinical application. Developing novel strategies that target gut microbial metabolites may yield promising results in improving patient outcome.

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Abbreviations

AhR	Aryl hydrocarbon receptor
AUC	Area under the curve
CRC	Colorectal cancer
DCA	Deoxycholic acid
FFAR2	Free fatty acid receptor 2
5-FU	5-Fluorouracil
ICIs	Immune checkpoint inhibitors
IFN	Interferon
IL	Interleukin
isoDCA	3β-Hydroxydeoxycholic acid
NK	Natural killer
Th	Helper T cells
T_{reg}	Regulatory T cells
SBAs	Secondary bile acids
SCFAs	Short-chain fatty acids
TMAO	Trimethylamine N-oxide
TME	Tumor microenvironment

Author contributions

YL researched data for the article, designed the figures, and wrote the manuscript. HCHL revised the figures and manuscript. JY supervised the study and revised the figures and manuscript.

Data availability statement

Data is available within the article or its supplementary materials.

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