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Part 1: Gut Microbiome and Cancer

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Abstract

The gut microbiome resides in the human gastrointestinal tract and has many roles in health and disease. This review discusses the effects of the gut microbiome on cancer. The gut microbiome can have either positive benefits or a negative impact on cancer progression and cancer therapy.

Keywords: Microbiome, Microbiota, Cancer, Bacterial metabolites, Cancer immunotherapy

Introduction

The gut microbiome in humans refers to a community of microorganisms in the digestive system. It is estimated that trillions of micro-organisms reside in the human gastro-intestinal tract, and the gut microbiome is considered to be an "organ", with similar metabolic activities or functions to other organs.[1] The gut microbiome plays many important roles in human physiology.[2,3] Many studies have shown that the gut microbiome and its metabolites have significant effects on a range of human diseases, such as inflammation and cancer,[4] and metabolic and cardiovascular diseases.[5] Short-chain fatty acids are metabolites that have many roles in human health and disease. Butyrate (**1**) (Figure 1) is produced by the gut microbiome through saccharolytic fermentation of dietary fibers, and improves insulin response in patients with type 2 diabetes.[6] Nicotinamide (**2**) (Figure 1) is produced by the human gut bacterium *Akkermansia muciniphila*, and improves motor-neuron function in mice and protects against progression of the neuro-degenerative disease amyotrophic lateral sclerosis (ALS) in mice.[7] It has been shown that the levels of nicotinamide (**2**) in cerebrospinal fluid of ALS patients are lower than in patients without ALS.[7] These studies have suggested that the gut microbiome could provide essential nicotinamide (**2**) and contribute to development and function of the nervous system in humans.

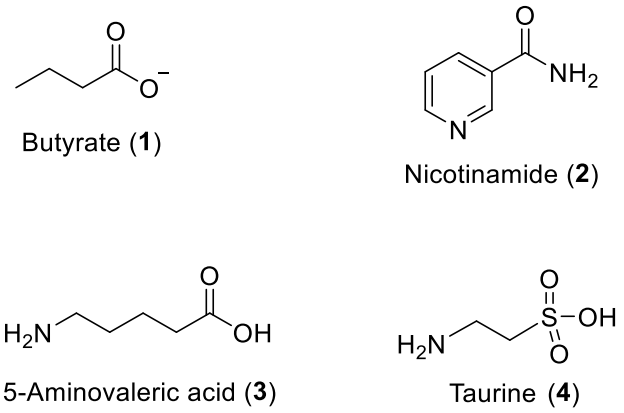


Figure 1. Structures of butyrate (**1**), nicotinamide (**2**), 5-aminovaleric acid (**3**), and taurine (**4**).

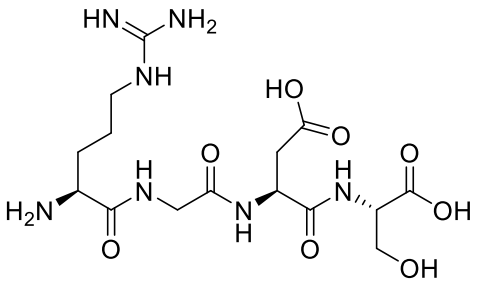
Several metabolites produced by the gut microbiome of patients with autism spectrum disorder have been shown to modulate autistic behavior in mice.[8] Autism spectrum disorder encompasses developmental disabilities with difficulties in social communication and interaction. In a mouse model, $GABA_A$ receptor agonists 5-aminovaleric acid (**3**) and taurine (**4**) (Figure 1) were produced by gut bacteria and modulated behaviors associated with autism spectrum disorder.[8] In comparison with the control group, mice supplemented with 5-aminovaleric acid (**3**) and taurine (**4**) had significant improvement of repetitive and social behaviors.[8] The gut microbiota has been linked with other diseases. Imbalance of gut microorganisms, known as dysbiosis, may lead to several diseases, including different types of cancer.[9] The relationship between gut microbiota and cancer is discussed in this review. We provide research evidence of the negative impacts of the gut microbiome on cancer, and we describe the positive impacts of the gut microbiome on cancer therapy. This review focuses only on gut bacteria and not other microorganisms, such as fungi and yeasts.

Negative Impacts of Gut Microbiome on Cancer

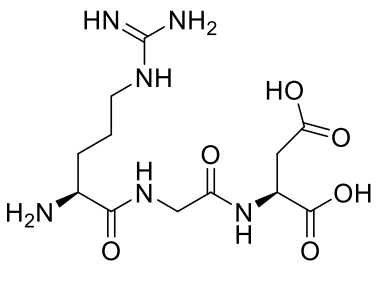
The gut microbiome resides in the human gastrointestinal tract and is closely related to colorectal or colon cancer. Colorectal cancer caused 881,000 deaths worldwide in 2018, and it is estimated that there are 1.8 million new cases annually.[10] The diversity and composition of the gut microbiome have a significant positive correlation with the development of colorectal cancer.[11] Although overall microbial compositions of colorectal cancer and noncancerous tissues are similar, the microbiome in colorectal cancer has lower microbial diversity. Moreover, microbial composition in the intestinal lumen is significantly different from that of colorectal cancer tissue.[11] It is suggested that the mucosa-associated microbiome contributes to the risk of colorectal cancer through direct interaction with the host; possibly via metabolic exchange or co-metabolism with the host.[11] Certain gut microorganisms are associated with colorectal cancer. The abundance of *Peptostreptococcus anaerobius*, an anaerobic bacterium, is significantly higher in stool samples of patients with colorectal cancer compared with that in people without colorectal cancer.[12] *P. anaerobius* has been shown to induce colon dysplasia (cancer-like cells) in a mouse model of colorectal cancer. A study of the mechanisms of carcinogenesis has revealed that *P. anaerobius* stimulates Toll-like receptors (e.g. TLR2 and 4) on colon cells and subsequently upregulates production of reactive oxidative species, thus stimulating cholesterol synthesis and cell proliferation.[12] Another study has revealed

that *P. anaerobius* alters the cancer immune microenvironment, leading to acceleration of colorectal carcinogenesis.[13] Transmission electron microscopy has shown that *P. anaerobius* selectively attaches to colorectal cancer cell lines rather than normal colon epithelial cells. Putative cell wall binding repeat (PCWBR)2 is a surface protein of *P. anaerobius* that interacts with a receptor, α_2/β_1 integrin, which is overexpressed on colorectal cancer cell lines, thus activating the PI3K–Akt pathway via phospho-focal adhesion kinase. Interaction of PCWBR2 and α_2/β_1 integrin can increase activation of nuclear factor kappa-light-chain-enhancer of activated B cells and cell proliferation, thus promoting progression of colorectal cancer.[13] Peptide RGDS (**5**), which is a derivative of arginylglycylaspartic acid (RGD) (**6**) (Figure 2), blocks the interaction between PCWBR2 and α_2/β_1. This abolishes the oncogenic response mediated by *P. anaerobius* and host cell interaction *in vitro* and *in vivo*.[13] The RGD can bind to many proteins, such as fibronectin, and facilitate bacterial cell attachment to the host cells via integrins, which are a family of cell-surface proteins.[14]

The gut microbiome is also associated with recurrence of colorectal cancer and failure of chemotherapy. The gut bacterium *Fusobacterium nucleatum* has been found to contribute to chemoresistance in mice, and it is pathologically associated with cancer recurrence in patients[15] *F. nucleatum* can activate TLR4 and myeloid differentiation primary response 88 of host cells to initiate innate immune signaling. *F. nucleatum* also targets specific miRNAs and activates the autophagy pathway. The interaction of *F. nucleatum* and TLRs, miRNAs, and autophagy network could reduce the response of colorectal cancer to chemotherapy. Therefore, it has been suggested that treatment of colorectal cancer should be not only with conventional chemotherapy, but also with antibiotics to suppress *F. nucleatum* or with supplementation of an autophagy inhibitor.[15] *F. nucleatum* can stimulate progression of colorectal cancer by inducing inflammation and host immune response in the colorectal cancer microenvironment.[16] Bacterial surface proteins FadA, Fap2 and RadD adhere to human intestinal epithelium, and stimulate the host cells to produce inflammatory mediators, including cytokines, for recruitment of inflammatory cells. This adhesion process provides a microenvironment that stimulates growth of colorectal cancer cells.[16] *F. nucleatum* is able to suppress the functions of immune cells such as macrophages, T cells and natural killer cells, and this immu-nosuppression can promote colorectal cancer.[16]



RGDS (**5**)



RGD (**6**)

Figure 2. Structures of peptides RGDS (**5**) and RGD (**6**).

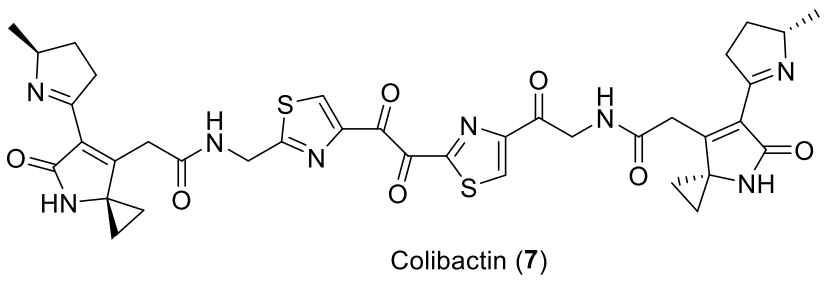


Figure 3. Structure of colibactin (**7**).

Escherichia coli is a pathogenic member of the Enterobacteriaceae that is considered to be associated with colorectal cancer. Patients with colorectal cancer have higher numbers of *E. coli* in their colonic mucosa compared with healthy people. Analysis of the colonic mucosa of patients with colorectal cancer and inflammatory bowel disease has revealed that these *E. coli* carry a *pks* genomic island, which contains genes encoding colibactin (**7**) biosynthesis (Figure 3).[17] Changes in the composition of gut bacteria or introducing genotoxic microorganisms is associated with tumorigenesis. It has been observed in a mouse model that deletion of polyketide synthase in *E. coli* reduces progression of colorectal cancer without altering intestinal inflammation.[17] The *pks* genomic island in *E. coli* regulates cellular processes that enhance cancer cell growth, suggesting that colibactin (**7**) is involved in the progression of cancer cells.[18] Colibactin (**7**) is therefore a genotoxic secondary metabolite of *E. coli* that causes colorectal cancer. Other bacterial species such as *Enterobacter aerogenes*, *Klebsiella pneumoniae*, and *Citrobacter koseri* also have the *pks* gene.[19] Colibactin (**7**) was first reported in 2006, and this bacterial metabolite can induce DNA double-strand breaks.[20] However, there are many colibactin derivatives, which are collectively known as colibactins.[21] It is known that colibactins can form DNA crosslinks by alkylation of adenine residues, thus leading to tumorigenesis, and these metabolites of the gut microbiome are harmful to humans.

Among several colibactins, colibactin (**7**) (Figure 3) is the genotoxic secondary metabolite that has received the most research attention, and its structure was established in 2019.[22] The structure of colibactin (**7**) was difficult to determine because *E. coli* produces it at low levels. Precolibactins are also bacterial metabolites produced by commensal *E. coli*, and both precolibactins and colibactins are encoded by a gene cluster of hybrid polyketide synthase–nonribosomal peptide synthetase (PKS-NRPS).[23] Precolibactins are considered to be nontoxic metabolites; however, the enzyme colibactin peptidase transforms precolibactins to genotoxic colibactins by cleavage of an *N*-acyl-D-asparagine side chain.[24] Evaluation of the ability of DNA binding and alkylation activity of colibactin derivatives, such as compound (**8**) (Figure 4), has revealed that imine, unsaturated lactam, and cyclopropane functionalities can potently alkylate DNA, but the pyridone group is not involved in DNA alkylation.[23] Among these functionalities, the cyclopropane moiety has received a lot of attention. The protein colibactin self-protection protein (ClbS; previously known as c2450), encoded by the *clbS* gene converts colibactin derivative (**9**) to hydroxyfuran derivative (**10**), and the X-ray structure and molecular function of ClbS were studied by Tripathi and co-workers.[25] The cyclopropane moiety is responsible for DNA alkylation, and Xue and co-workers revealed the structure of colibactin-nucleobase adduct using colibactin derivative (**11**) as a substrate.[26] The cyclopropane- opened

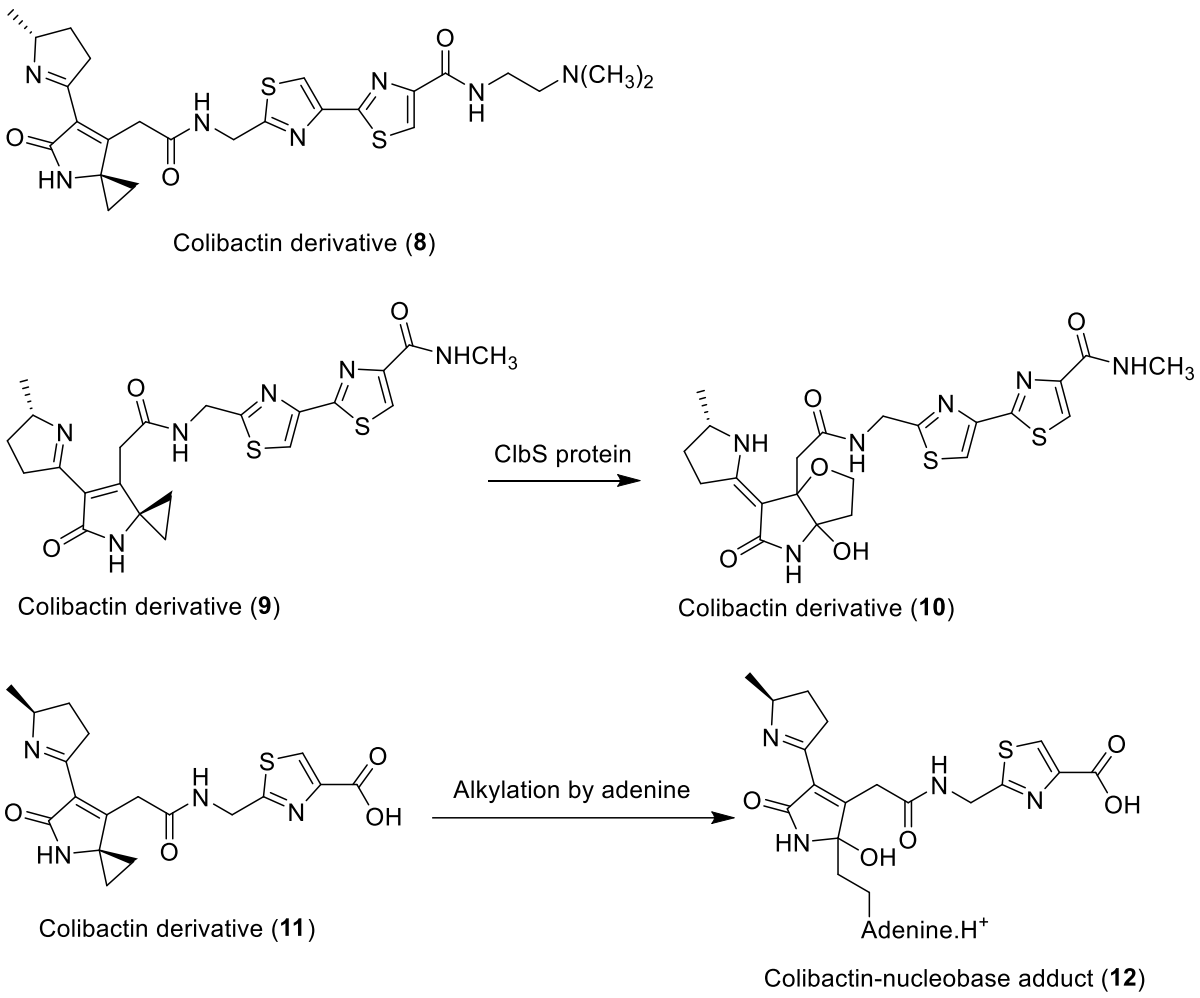


Figure 4. Structures of colibactin derivatives (**8**–**11**), and colibactin-nucleobase adduct (**12**).

product was proposed as colibactin-nucleobase adduct (**12**); adenine nucleobase attacked the cyclopropane moiety of colibactin derivative (**11**), giving rise to the adduct (**12**) (Figure 4).[26] However, this experiment used mass spectrometry (MS) for the identification of the structure of colibactin-nucleobase adduct (**12**); therefore, the position of the adenine nucleobase attached to colibactin could not be assigned.

The colibactin-nucleobase adducts have received attention from scientists worldwide. In 2019, the structures of colibactin-nucleobase adducts were finally elucidated by Wilson and co-workers.[27] The cyclopropane group in colibactins is considered to be an electrophilic warhead that readily reacts with DNA by alkylation. Incubation of colibactin derivative (**13**) (Figure 5) with calf-thymus DNA for 20 h at 37 ℃ gave only trace amounts of detectable adducts, and slightly cleaved DNA was observed at 1 mM concentration of colibactin derivative (**13**). This was because a carboxylic functionality in colibactin derivative (**13**) interacted electrostatically interaction with a phosphate backbone of DNA that was negatively charged in the molecule.[27] Therefore, an ethyl ester derivative, compound (**14**) (Figure 5), was used for experiments of DNA alkylation, giving ~100 times more potency than colibactin derivative (**13**) in a DNA shearing assay. Moreover, colibactin derivative (**14**) could induce both G2/M cell cycle arrest and DNA double-strand breaks in HeLa cells.[27]

Liquid chromatography-MS analysis of the colibactin-adenine adducts revealed a mixture of two diastereomeric adducts of (**15**) (Figure 5), as indicated by two peaks on the chromatogram. ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy also demonstrated signals of two diastereomeric adducts. Colibactin-adenine adduct (**15**) was hydrolyzed by pig liver esterase, yielding colibactin-adenine adduct (**16**) (Figure 5). Key long-range correlations of ^{1}H to ^{13}C observed in the heteronuclear multiple bond nuclear magnetic resonance technique revealed that a 5-hydroxypyrrolidin-2-one ring system was attached to an *N3*-substituted adenine ring (Figure 5).[27] Although the structure of colibactin-adenine adducts (**15**) and (**16**) is well characterized, they are synthetic derivatives of colibactins. Attempts have been made for structural elucidation of natural colibactin produced by *E. coli*, and finally the structure of colibactin (**7**) (Figure 3) was recently established by Xue and colleagues.[22]

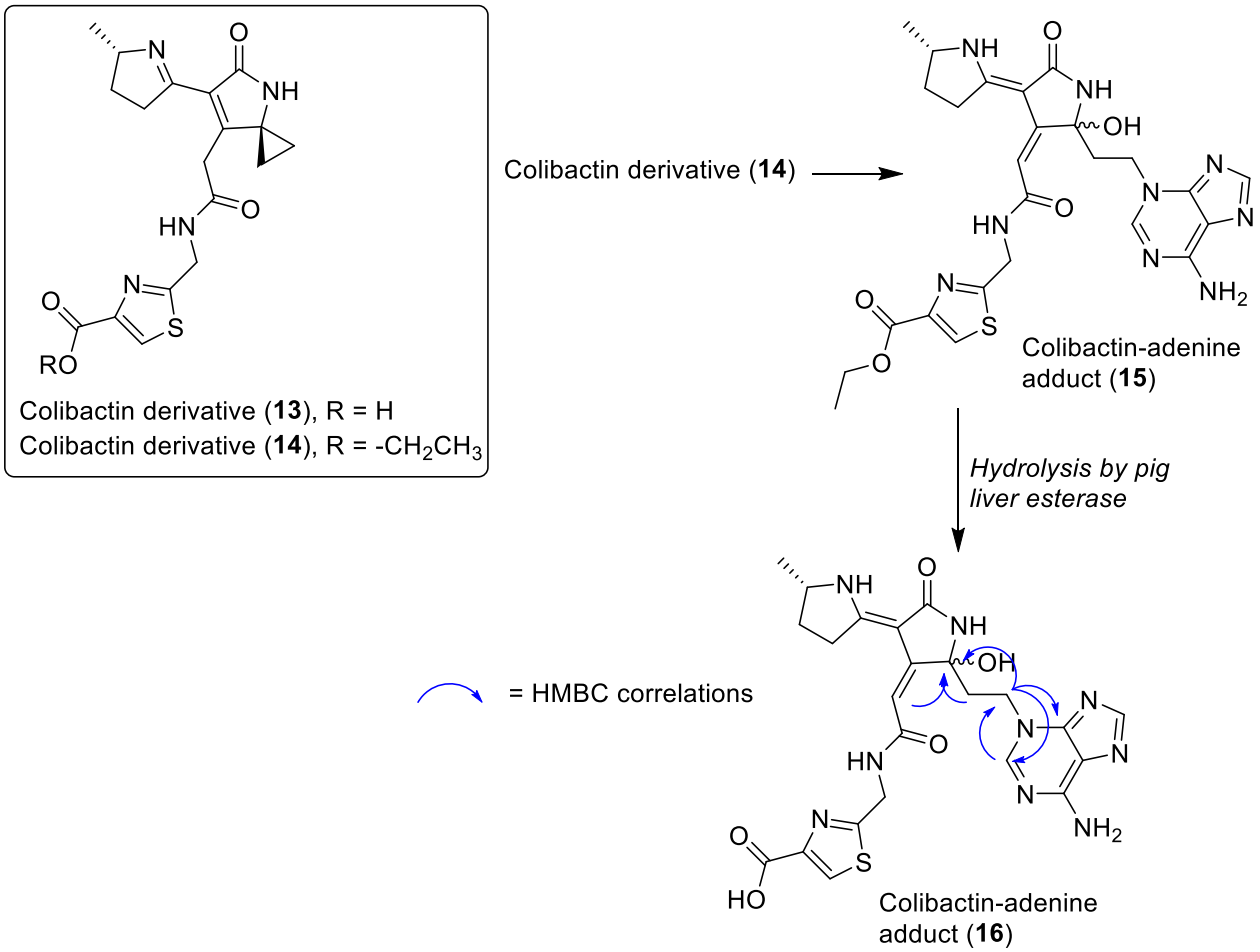


Figure 5. Structures of colibactin derivatives (**13**) and (**14**), colibactin-adenine adducts (**15**) and (**16**), and heteronuclear multiple bond (HMBC) correlations of (**16**).

Positive Impacts of the Gut Microbiome on Cancer

The gut microbiome improves the therapeutic efficacy of some anticancer drugs. Co-administration of antibiotics and anticancer drugs may lead to poor efficacy of anticancer drugs because the antibiotics suppress the growth and alter the profile of the gut bacteria. The gut microbiome plays an important role in modulating efficacy of immune checkpoint inhibitors (ICIs), by targeting the programmed cell death receptor/ligand-1 (PD-1/PD-L1) pathway.[28] Co-administration of antibiotics and ICIs was studied in a cohort of 109 Chinese patients with advanced non-small cell lung cancer. The patients treated with antibiotics and ICIs had shorter survival time compared with the control group treated with ICIs alone. Multivariable analysis has revealed that antibiotic treatment is markedly associated with worse progression-free survival of patients.[28] It is proposed that co-administration of antibiotics might be responsible for dysbiosis (or dysbacteriosis), thus attenuating the clinical outcomes of patients who have received immunotherapy. Dysbiosis is an imbalance in the microflora in the human gastrointestinal tract, which leads to changes in composition of the gut microbiome and metabolic activities. The study by Zhao and co-workers highlighted the significance of gut microbiome composition for successful cancer therapy.[28]

The impact of the gut microbiome on cancer immunotherapy has been investigated in animal models. The gut bacterial genus *Bifidobacterium* was associated with enhancement of efficacy of anticancer drugs in animals.[29] Oral administration of *Bifidobacterium* to mice improved cancer immunotherapy with PD-L1-specific antibody,[29] indicating that gut microbes enhance the therapeutic benefit of cancer immunotherapy. A study in humans by Matson and co-workers revealed a significant association between the gut microbiome and anti-PD-1 efficacy in metastatic melanoma patients.[30] *Bifidobacterium longum*, *Collinsella aerofaciens*, and *Enterococcus faecium* were found to significantly improve anticancer drug response.[30] Furthermore, greater efficacy of anti-PD-L1 therapy was observed in germ-free mice supplemented with fecal material from patients with good anticancer drug response. This study demonstrated the mechanistic impact of the gut microbiome on antitumor immunity in cancer patients.[30]

In summary, gut microorganisms play important roles in cancer, and they can have positive or negative impacts. Some gut bacteria can produce natural metabolites that cause cancer in humans, whereas other gut bacteria enhance the efficacy of cancer immunotherapy. Better understanding of the interactions between the gut microbiome and the host may lead to new diagnostic methods and treatment for cancer.

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