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BASIC SCIENCE FOUNDATIONS IN COLORECTAL CANCER (J ROPER, SECTION EDITOR)

# Gut Microbiome and Colon Cancer: Role of Bacterial Metabolites and Their Molecular Targets in the Host

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**Abstract**
*Purpose of Review* The relationship between colonic bacteria and the host is symbiotic, but how communication between the two partners occurs is just beginning to be understood at the molecular level. Here, we highlight specific products of bacterial metabolism that are present in the colonic lumen and their molecular targets in the host that facilitate this communication.
*Recent Findings* Colonic epithelial cells and mucosal immune cells express several cell surface receptors and nuclear receptors that are activated by specific bacterial metabolites, which impact multiple signaling pathways and expression of many genes. In addition, some bacterial metabolites also possess the ability to cause epigenetic changes in these cells via inhibition of selective enzymes involved in the maintenance of histone acetylation and DNA methylation patterns.
*Summary* Colonic bacteria communicate with their host with selective metabolites that interact with host molecular targets. This chemical communication underlies a broad range of the biology and function of colonic epithelial cells and mucosal immune cells, which protect against inflammation and carcinogenesis in the colon under normal physiological conditions.

**Keywords** Bacteria-host symbiosis · Colonic inflammation · Colon cancer · Bacterial metabolites · Cell surface receptors · Nuclear receptors · Epigenetics · Histone deacetylases · TET DNA demethylases · HIF1α-prolylhydroxylases · NDRG3

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## Introduction

From the first day of life, we live in cohabitation with microorganisms in different parts of our bodies. Colonization of our bodies by these microorganisms begins at birth irrespective of the mode of delivery, even though the specific types of microorganisms that we encounter at birth may differ between vaginal and Cesarean section delivery. Until recently, it was thought that adult humans harbor approximately 10 times more bacterial cells than the human cells, primarily based on a four-decade-old estimate [1]. However, a recent study indicates that the ratio of bacterial cells to human cells in our body is significantly less, closer to a ratio of 1:1 [2••], with most of these bacteria residing in our large intestine.

Although the relationship between colonic bacteria and the host is often described as commensal, the relationship is actually symbiotic, with active communication between the bacteria and the host and both benefitting from the cohabitation. The benefits that the bacteria derive from the host are obvious; we provide the space for bacteria to colonize and also food for them to survive and grow. The benefits that the host derives from

cohabitation are just beginning to be understood. Normal bacteria in the colon protect against enteric infections, aid in the maturation of colonic function, modulate the gut mucosal immune system, supply certain vitamins, promote water and electrolyte absorption, influence the epigenetic profile of epithelial cells, and maintain energy homeostasis [3–6].

It would be incorrect to assume that cohabitation results in only beneficial outcomes for host health. Colonic bacteria also play critical, causal roles in the development of colonic diseases as well as in a variety of systemic diseases [7–10]. As such, the recognition of the importance of gut microbiota to our health has been one of the hallmarks of the last decade, underlined by the initiation of the Human Microbiome Project by the National Institutes of Health. The initial efforts of the Human Microbiome Project focused on sequencing and annotating individual microbial genomes, but the focus has recently shifted to understanding the molecular mechanisms underlying the effective communication between bacteria and host. Specifically, bacterial metabolites are released into the colonic lumen, which then function as messengers between the bacteria and the host. The purpose of the current review is to summarize recent discoveries related to these bacterial metabolites and their molecular targets in the host and to highlight the significance of this chemical communication to host health and disease.

## Bacterial Metabolites as the Messengers in the Communication Between Colonic Bacteria and the Host

Colonic bacteria release specific metabolites via multiple metabolic pathways into the colonic lumen. The normal turnover of bacteria also releases metabolic intermediates from the dead cells into the colonic lumen. Metabolite production and release depends on various factors, including the composition of the host diet and the bacterial strains that colonize the colon. Most of these metabolites are likely to have only local effects on the host cells in the vicinity of the bacteria (e.g., colonic epithelial cells, mucosal immune cells, enteric neurons), but some of these metabolites may enter the systemic circulation and elicit biological effects on cell types distant from the bacteria [11•]. The impact of the colonic bacteria and their metabolites on distant organs could also be due to mucosal immune cells in the gut lamina propria, which are biologically and immunologically altered and then travel to distant sites [12, 13••]. The circulating bacterial metabolites and immune cells, which are trained and programmed in the intestinal tract, provide a mechanistic connection between gut microbiota and systemic diseases such as diabetes, obesity, metabolic syndrome, atherosclerosis, autism, autoimmunity, and cancer [14–19].

## Identity of Bacterial Metabolites and Their Molecular Targets Relevant to Bacteria-Host Interaction

Table 1 lists bacterial metabolites in the colonic lumen that have been shown to induce significant biological effects locally on the colonic epithelial cells and mucosal immune cells as well as distantly on other organs. Table 1 also identifies respective host molecular targets for these metabolites. We have placed these metabolites in four groups, primarily based on their structure and/or origin. Group 1 (short-chain carboxylates) consists of the monocarboxylates acetate, propionate, butyrate, and lactate, as well as the dicarboxylate succinate. These are generated in the colonic lumen principally by bacterial fermentation of dietary fiber, but some of these are also found in normal diet (e.g., lactate in yogurt). The molecular targets for these metabolites include cell surface G-protein-coupled receptors (GPR41, GPR43, GPR109A, GPR81, and GPR91), intracellular enzymes (HDACs, TETs, and PHD2), and an intracellular signaling component (NDRG).

Group 2 consists of tryptophan metabolites (indole, indole-3-aldehyde, indole-3-acetic acid, and indole-3-propionic acid). These metabolites are generated by colonic bacteria via metabolism of tryptophan synthesized by bacteria or from dietary sources. Their molecular targets are selective nuclear receptors (AhR and PXR).

Group 3 represents lipid metabolites arising from endogenous lipids in bacteria and/or dietary lipids. This includes the

**Table 1** Bacterial metabolites and their molecular targets in the host

| Metabolites | Targets |
|---|---|
| Short-chain carboxylates | |
| Acetate, propionate, butyrate | GPR41, GPR43, HDACs |
| Butyrate | GPR109A, HDACs |
| Lactate | GPR81, NDRG3 |
| Succinate | GPR91, TETs, PHD2 |
| Tryptophan metabolites | |
| Indole, indole-3-aldehyde | AhR |
| Indole-3-acetic acid | AhR |
| Indole-3-propionic acid | PXR |
| Lipids and lipid metabolites | |
| Trimethylamine | TAAR5 |
| Deoxycholic acid, lithocholic acid | FXR, PXR |
| Conjugated linoleic and linolenic acids | PPARα, PPARγ |
| 10-Hydroxy-*cis*-12-octadecenoate | GPR40 |
| Bacterial cell wall components | |
| Lipopolysaccharide | TLR4 |
| Peptidoglycans | NOD1, NOD2 |
| Polysaccharide A | TLR2 |

organic cation trimethylamine (TMA), secondary bile acids deoxycholic acid and lithocholic acid, and modified polyunsaturated fatty acids. In host cells, these lipid metabolites signal through nuclear receptors (FXR, PXR, PPARα, and PPARγ) as well as cell surface receptors (GPR40 and TAAR5). Group 4 is primarily comprised of bacterial cell wall components (lipopolysaccharide, peptidoglycans, and polysaccharide A). These metabolites act through cell surface receptors (TLR4) and intracellular receptors (TLR2, NOD1, and NOD2).

## Short-Chain Carboxylates: Short-Chain Fatty Acids

Colonic bacteria produce high concentrations of short-chain carboxylates in the lumen, which include the short-chain fatty acids acetate, propionate, and butyrate, the glycolysis product lactate, and the citric acid cycle intermediate succinate. Among these, the short-chain fatty acids have been most extensively investigated for their effects on the colon. Colonic bacteria ferment carbohydrates and generate acetate, propionate, and butyrate as their major end products. Dietary fiber constitutes the majority of these fermentable carbohydrates. The combined luminal concentrations of these three fermentation products are in the range of 50–100 mM, with an approximate ratio of 6:3:1 for acetate, propionate, and butyrate, respectively. The beneficial effects of these short-chain fatty acids on colonic health have been known for several decades, but a majority of investigations in the area focused on butyrate as an inhibitor of histone deacetylases (HDACs) [20, 21]. Recent studies show that propionate is also an inhibitor of HDACs [22, 23], that butyrate functions selectively as an agonist for the cell surface G-protein-coupled receptor GPR109A (also known as hydroxycarboxylic acid receptor 2 or HCAR2) [24], and that all three short-chain fatty acids serve as agonists for two other cell surface G-protein-coupled receptors, identified as GPR41 and GPR43 [25–27].

There are two transporters in the colonic epithelium, expressed in the lumen-facing apical membrane, that mediate the uptake of short-chain fatty acids from the lumen: the $H^+$-coupled monocarboxylate transporter MCT1 (SLC16A1) and the $Na^+$-coupled monocarboxylate transporter SMCT1 (SLC5A8) [28, 29]. It is logical to presume that any transport mechanism involved in the uptake of an HDAC inhibitor into cells would suppress carcinogenesis because HDACs are upregulated in many cancers and HDAC inhibitors are effective in cancer therapy. However, a tumor-suppressive function has been unequivocally demonstrated only for SMCT1, not for MCT1 [30, 31]. In fact, the tumor-suppressive role of SMCT1 in the colon was discovered first [32], followed by the discovery of the functional identity of the transporter as the protein responsible for the $Na^+$-coupled high-affinity uptake of short-chain fatty acids (including butyrate and propionate) in colonic epithelial cells [33]. Despite the ability of SMCT1 to function as a tumor suppressor in vitro, mice lacking the transporter do not demonstrate a significantly increased risk for cancer [34]. The tumor-suppressive function of the transporter is linked to the fiber content in the diet; even though the knockout mice did not show any difference in cancer risk when fed a diet rich in fiber, the mice exhibited increased cancer risk when fed a diet low in fiber [35••]. This finding appears to be directly linked to the high affinity of SMCT1 for butyrate and propionate, which makes the contribution of the transporter to the total uptake of these HDAC inhibitors in colonic epithelial cells insignificant when these metabolites are present at high concentrations, as in the setting of a high-fiber diet.

In addition to their ability to influence the epigenetic profile of colonic epithelial cells, propionate, butyrate, and acetate also impact epithelial cell function by activating three different cell surface receptors: GPR109A, GPR43, and GPR41 [25–27]. GPR109A (also known as hydroxycarboxylic acid receptor 2 or niacin receptor 1), a selective receptor for butyrate, is expressed on the lumen-facing apical membrane of colonic epithelial cells; its expression, together with butyrate, increases along the intestinal tract from jejunum to colon [36]. Interestingly, colonic bacteria regulate the expression of *HCAR2* (the gene encoding GPR109A) in the colon [36]; gene expression is markedly reduced in germ-free mice and returns to normal when the colons of the germ-free mice are colonized with bacteria. Activation of GPR109A elicits anti-inflammatory and tumor-suppressive effects. Conversely, *Gpr109a*-null mice are more prone to experimental colitis and colon cancer. This study also showed that the butyrate receptor is expressed on mucosal immune cells and that the receptor on these immune cells as well as the receptor on colonic epithelial cells contribute to the protection against inflammation and carcinogenesis in the colon [37••].

The participation of immune-cell GPR109A in the maintenance of colonic health raises a critical question: what is the agonist for this receptor on the immune cells in the lamina propria? It is unlikely that the bacteria-derived butyrate would cross the colonic epithelial cells to the serosal side at concentrations high enough to activate the receptor on immune cells. Taggart et al. have shown that the ketone body β-hydroxybutyrate is the physiological agonist for GPR109A expressed in non-colonic tissues [38]. It remains to be determined if activation of the immune-cell GPR109A by circulating β-hydroxybutyrate is responsible for the contributions of the receptor signaling to colonic health. Despite the current lack of information on the identity of the agonist for the immune-cell GPR109A in the lamina propria of the colon, strong evidence exists for a critical role for this receptor in mucosal immune function. A recent study showed that GPR109A expressed on immune cells might function to enhance oral tolerance and protect against food allergy [39••].

The roles of GPR41 and GPR43, which are activated by all three short-chain fatty acids, remain unclear [40]. GPR41 is expressed in colonic epithelial cells as well as in enteroendocrine cells, [41] whereas GPR43 is expressed in colonic epithelial cells, enteroendocrine cells, and immune cells [42, 43]. However, based on published literature, it is difficult to determine whether the signaling pathways associated with these two receptors are anti-inflammatory or pro-inflammatory. For example, Trompette et al. [44] reported that *Gpr41*-null mice display increased inflammation, whereas Kim et al. [45] showed that *Gpr41*-null mice have reduced inflammation. The same is true with GPR43, with some studies showing evidence of anti-inflammatory function of the receptor [46–50] and other studies showing the opposite [45, 51].

## Short-Chain Carboxylates: Lactate and Succinate

Lactate is present in the colonic lumen at significant concentrations; it can arise from metabolism by different strains of bacteria, particularly *Lactobacilli*, and also can come from diet (e.g., yogurt). Until recently, lactate was considered merely a metabolic end product with relevance to energy production and also a carbon source for gluconeogenesis, but was recently recognized as an important signaling molecule [52]. This recognition stems from the discovery that lactate is an agonist for the cell surface G-protein-coupled receptor GPR81 [53]. However, there are no published reports on the expression of GPR81 in the intestinal tract. GPR81 is not the only mode of action for lactate; Lee et al. [54] recently identified NDRG3 (N-myc downstream regulated gene 3), an intracellular signaling molecule targeted by lactate that activates downstream signaling pathways involving RAF and ERK. Bacteria-derived lactate and dietary lactate enter colonic epithelial cells via the $H^+$-coupled and $Na^+$-coupled monocarboxylate transporters expressed on the apical membrane of these cells, but it is not known whether the newly discovered intracellular target NDRG3 is expressed in colonic epithelial cells and contributes to the biological effects of lactate.

Succinate is an additional bacterial metabolite present in the colonic lumen [55]. This metabolite is an inhibitor of the TET family of DNA demethylases and HIF1α prolyl hydroxylases (PHDs) [56, 57]; as such, succinate has the potential to impact the epigenetic profile of colonic epithelial cells by enhancing DNA methylation and to affect hypoxia signaling by increasing the cellular levels of the transcription factor HIF1α. However, there is minimal information on the relevance of succinate to colonic health. Succinate can also affect cellular function via the cell surface G-protein-coupled receptor GPR91 [58]. Similar to NDRG3, it is not known whether GPR91 is expressed in colonic epithelial cells and plays any role in the biology of these cells.

## Tryptophan Metabolites

A variety of tryptophan metabolites (e.g., indole, indole-3-aldehyde, indole-3-acetic acid, and indole-3-propionic acid) are generated primarily by bacterial metabolism in the colon and are then absorbed and circulated systemically [59, 60]; thus, plasma levels of these bacterial products are substantially lower in germ-free mice compared to conventional mice [61]. The nuclear receptors AhR (aryl hydrocarbon receptor) and PXR (pregnane/xenobiotic responsive receptor or pregnane X receptor) are molecular targets of these bacteria-derived tryptophan metabolites [59, 60, 62]. The activation of AhR by bacterial-derived tryptophan metabolites elicits anti-inflammatory and tumor-suppressive effects in the colon [63–66]. PXR also regulates cell proliferation, metastasis, and inflammation [67]; as such, bacterial metabolites of tryptophan that activate this receptor may affect the development and severity of colonic inflammation and colon cancer.

## Lipids and Lipid Metabolites

Colonic bacteria metabolize bile acids that escape enterohepatic circulation and enter the colon. Hepatocytes synthesize cholic acid and chenodeoxycholic acid, both of which are called “primary” bile acids, but a small portion of these bile acids reaches the colon and is converted into deoxycholic acid and lithocholic acid, respectively. This conversion occurs because of the ability of specific bacteria in the colon to remove the hydroxyl group in cholic acid and chenodeoxycholic acid at position 7 (i.e., 7α dehydroxylation); these bacteria-modified bile acids are called “secondary” bile acids. Colonic epithelial cells are directly exposed to these bile acids. These bile acids are absorbed into the portal circulation, taken up by hepatocytes, and re-secreted in bile. Normal bile therefore contains not only the primary bile acids but also the bacteria-derived secondary bile acids. Hepatocytes in the liver and absorptive epithelial cells in the ileum are exposed to the bacteria-generated bile acids. Bile acids are not only detergents but also signaling molecules that act through the nuclear receptors pregnane X receptor (PXR) and farnesoid X receptor (FXR) [68]. Bile acids, both primary and secondary, are required for the digestion and absorption of dietary fat and fat-soluble vitamins. In addition, these bile acids affect gene expression in target cells through PXR/FXR-mediated signaling. As such, colonic bacteria influence the biology of intestinal and colonic epithelial cells and hepatocytes. Other lipid metabolites that are generated in the colonic lumen by bacteria include conjugated linoleic and linolenic acids and 10-hydroxy-*cis*-12-octadecenoate; the molecular targets for these metabolites are the nuclear receptors PPARα and PPARγ, and the cell surface G-protein-coupled receptor GPR40.

It is generally assumed that normal colonic bacteria enhance colonic health and that, as the cohabitation of host and bacteria has evolved over millions of years, the symbiotic relationship does not harm either of the partners in the relationship. Therefore, it came as a surprise when recent studies provided evidence disputing this widely held notion. Colonic bacteria metabolize dietary lipids into trimethylamine, which is then converted in the liver into a potent cardiovascular toxin, trimethylamine N-oxide (TMAO) [69••, 70•]. Dietary choline and carnitine are the precursors for the bacterial generation of trimethylamine. Choline is normally found in the diet in the form of phosphatidylcholine (lecithin) and carnitine is a significant constituent of meat. TMAO is associated with atherosclerosis and cardiac events such as myocardial infarction, which provides a molecular link between the diets rich in fat and red meat and increased risk for atherosclerosis [69••, 70•]. The bacterial origin of this cardiovascular toxin became more evident from a recent study in mice that showed that the risk for atherosclerosis could be transmitted through gut microbial transplantation [71]. TMAO enhances platelet hyper-reactivity, activates macrophages, increases the transfer of cholesterol from the liver to peripheral tissues including the vasculature, and decreases the transfer of cholesterol from the periphery to the liver [16, 72, 73]. The molecular target for this cardiovascular toxin, however, remains unknown. The bacterial product TMA, which is the precursor for TMAO, is an agonist for the trace amine-associated receptor TAAR5 [74], but the biological relevance of the activation of this receptor in the colon and liver, the potential target organs for this bacterial metabolite, remains elusive.

## Bacterial Cell Wall Components

In addition to the metabolites generated by colonic bacteria, constituents of the bacterial cell wall also serve as signaling molecules in the host. These constituents include lipopolysaccharide, peptidoglycans, and polysaccharide A. The molecular targets for these bacterial cell wall components are present in colonic epithelial cells as well as in mucosal immune cells. These targets play essential roles in the function of the mucosal immune system. The Toll-like receptor 4 (TLR4), which is activated by lipopolysaccharide, is a cell surface receptor. Polysaccharide A activates peptidoglycans and TLR2, which in turn activate the intracellular receptors NOD1 and NOD2 (nucleotide-binding domain-containing proteins 1 and 2). These intracellular targets access peptidoglycans or polysaccharide A either via active mechanisms that uptake these ligands or by endocytosis/phagocytosis of the bacteria and subsequent generation of the ligands in the lysosomes following their fusion with endosomes/phagosomes.

Lipopolysaccharides (LPS), also known as endotoxins, constitute the major component of the outer membrane of Gram-negative bacteria. LPS exerts a broad range of biological effects on the intestinal tract, including the colon. It affects intestinal immune response, mucosal cell growth, energy metabolism, nutrient absorption, and mucosal barrier function. For example, it inhibits the absorption of the water-soluble vitamin biotin in the colon by interfering with the translocation of the transporter responsible for absorption to the apical membrane of colonic epithelial cells [75]. It also appears to promote the survival of enteric neurons, thus impacting intestinal motility [76]. The influence of LPS on enteric neurons might, however, depend on diet; high-fat diet induces dysmotility through degeneration of enteric neurons [77]. It also plays a critical role in intestinal neoplasia; colorectal cancer is associated with overexpression of LPS signaling [78, 79]. Although TLR4 is considered to be the principal cell surface receptor for LPS, other proteins are also involved in LPS-mediated signaling, both in a TLR4-dependent and TLR4-independent manner. The LPS receptor is a multimeric complex, consisting of at least three proteins: TLR4, CD14, and MD2. CD14 is a membrane-associated protein, anchored to the outer surface of the membrane via the lipid glycosylphosphatidylinositol. It binds LPS and is capable of intracellular signaling independent of TLR4; it also plays a role in presenting LPS to TLR4/MD2 complex to elicit TLR4-dependent intracellular signaling [80, 81]. Of biological importance is the finding that three components of the LPS receptor complex exhibit a differential expression pattern depending on the cell type and the animal species. Normal human colonic epithelial cells express CD14 but not TLR4, whereas normal mouse colonic epithelial cells express both [82••]. In contrast, monocytes, macrophages, and dendritic cells express all three components, in humans as well as in mice [83].

Interestingly, the biological outcome of LPS signaling in colonic epithelial cells varies depending on whether or not TLR4 is co-expressed with CD14. In human colonic epithelial cells, which lack TLR4, the binding of LPS to CD14 causes cell death and suppresses carcinogenesis in a phospholipase C- and sphingomyelinase-dependent manner, whereas the co-expression of TLR4 and CD14 in murine colonic epithelial cells promotes cell survival and carcinogenesis in response to LPS in an NF-κB-dependent manner [82••]. Human colon cancer tissues as well as most colon cancer cell lines of human origin express both TLR4 and CD14, thus suggesting a role for LPS in promoting proliferation of cancer cells [82••]. As murine colonic epithelial cells normally co-express TLR4 and CD14, overexpression of TLR4 specifically in colonic epithelial cells increases cell proliferation, promotes development of longer colonic crypts, expands Lgr5-positive crypt cells, and potentiates carcinogenesis [84]. There is evidence that LPS-induced signaling via the TLR4/CD14 complex cross-reacts with β-catenin/Wnt pathway to promote colonic neoplasia [84]. In addition, dendritic cells and neutrophils in the lamina

propria express acyloxyacyl hydrolase, an enzyme capable of inactivating LPS via deacylation of its lipid A moiety [85]. The outcome of LPS signaling via TLR4 in colon also depends on whether the exposure of the tissue to LPS is acute or chronic; acute exposure elicits pro-inflammatory effects via promotion of naïve T cell polarization towards Th17-positive cells, whereas chronic exposure elicits an opposite response, promoting immune tolerance via polarization of naïve T cells towards regulatory T cells (Tregs) [86•].

## Conclusions

Bacteria in the colon elicit mostly beneficial effects on the host, which include protection against inflammation and carcinogenesis. This phenomenon is principally driven by interactions between specific bacterial metabolites and their respective molecular targets in the host. These interactions inhibit inflammation and tumorigenesis as a result of a broad spectrum of signaling pathways encompassing genetic and epigenetic events. Thus, the assumption that colonic bacteria are silent partners in cohabitation with the host is no longer valid. There is active communication between the bacteria and the host, in both directions, that enables the symbiotic relationship to work effectively for the benefit of both partners.

#### Compliance with Ethical Standards

**Conflict of Interest** The authors declare that they have no conflict of interest.

**Human and Animal Rights and Informed Consent** All reported studies/experiments with human or animal subjects performed by the authors have been previously published and complied with all applicable ethical standards, including the Helsinki declaration and its amendments, institutional/national research committee standards, and international/national/institutional guidelines. The data reported as "unpublished" were from animal studies performed with approval from the Institutional Animal Care and Use Committee.

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