From microbe to man: the role of microbial short chain fatty acid metabolites in host cell biology

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IN RECENT YEARS, STUDIES have demonstrated a variety of ways in which microbiota, particularly in the gut, interact with and influence host physiology. For instance, it is now well established that changes in the composition and diversity of the gut microbial community are associated with obesity and/or with changes in diet (14–16, 41, 50, 76–78). In addition, gut microbiota have been linked to a wide range of changes in other physiological parameters and pathophysiological conditions, including renal function (59, 80, 81), cardiovascular function (58, 59), irritable bowel syndrome (13, 19), atherosclerosis (83), and immune disorders (2, 38, 43, 54). However, it is important to note that although it seems clear that there is a correlation between changes in gut microbiota and changes in host status (i.e., obesity), the mechanism by which these changes are linked is largely unknown. In addition, although it is well appreciated that host-microbe interactions play important roles in host physiology, in many cases we do not yet understand how these interactions occur at the cell signaling level. To truly understand the nature of these interactions, with the hope of potentially manipulating them therapeutically, we must understand the cell signaling pathways underlying these communications.

One way that commensal microbiota communicate with the host is through the generation of metabolites, which are then absorbed into the bloodstream and sensed by host G protein-coupled receptors (GPCRs). The most well-studied microbial metabolites to date are short chain fatty acids (SCFAs), the most abundant of which are acetate, propionate, and butyrate. SCFAs are produced by microbial fermentation of complex polysaccharides (starches and fiber) in the colon, and as a result the concentration of SCFAs in the colon is ~100 mM (8). Acetate, propionate, and butyrate are absorbed into the colonic epithelium, where some butyrate is utilized by colonocytes as an energy source before the remaining SCFAs are absorbed into the bloodstream of the host (34). SCFAs in the bloodstream of the host have been reported at concentrations ranging from 0.1 mM to 10 mM (49, 54, 61). Although there are other minor nonmicrobial sources of plasma SCFAs, the primary source of SCFAs in the bloodstream is gut microbial metabolism (8). Interest in SCFAs as microbe-to-host signals has steadily increased in recent years, as several studies linking gut microbes to host physiology have shown that SCFAs appear to be the “link” by which microbes communicate with the host (50, 54, 58, 62, 87, 91). For example, it was reported that gut microbiota-mediated effects on host adiposity are mediated by a host receptor for SCFAs (62). Therefore, it appears that SCFAs and SCFA-mediated cell signaling are key to further our understanding of host-microbe interactions. In this review, we will consider what is currently known regarding the proteins responsible for SCFA detection and transport on the cellular level, and then will discuss known effects of SCFAs on the cell physiology (Fig. 1). In the future, we hope that by combining our knowledge of whole animal physiology studies with an understanding of SCFA-mediated cell signaling, we will be able to obtain a fuller understanding of host-microbe interactions, and ultimately a better understanding of host physiology.

RECEPTORS AND TRANSPORTERS FOR SCFAS

To understand the physiological actions of SCFAs, we must consider the cellular mechanisms underlying the detection and transport of these compounds. In this portion of the review, we will introduce the proteins that are thought to play a role in sensing or transporting SCFAs.
SCFA Transporters

Initial reports of SCFA transport in the colon found that SCFA absorption was associated with increased Na\(^+\) uptake (4, 5); this Na\(^+\) dependency was begun to be understood when it was discovered that SLC5A8 functions as an apical transporter in the colon. SLC5A8 is a sodium-coupled monocarboxylate transporter (10, 28, 56), which is well-expressed in the apical membrane of the colon (40) where it transports butyrate transporter (10, 28, 56), which is well-expressed in the colon where it transports SCFAs, with acetate, propionate, and butyrate being the strongest ligands for Gpr41 and Gpr43 (7, 49). Gpr41 was found to couple to Gi (7, 49) and to be most responsive to propionate [EC\(_{50}\): Gpr41 = 12 \(\mu\)M; (49)], although a variety of other short-chain fatty acids including formate, acetate, butyrate, and isobutyrate elicit varying degrees of activation. Importantly, the human and rat orthologs of Gpr41 displayed similar SCFA response profiles (7), indicating that the signaling of this receptor is likely to be evolutionarily conserved. An additional receptor present in humans, Gpr42, has a very high level of homology to Gpr41, but it is unclear whether Gpr42 is a functional gene or a pseudogene (7, 52).

In the initial 2003 paper, Le Poul et al. (49) astutely pointed out that a subset of the stronger ligands for Gpr41 and Gpr43 (acetate, propionate, and butyrate) are “produced in considerable amounts by microbial fermentation in the hindgut.” Indeed, subsequent studies demonstrated that Gpr41 is expressed in a variety of tissues and cell types including the colon, kidneys, sympathetic nervous system, and blood vessels, (42, 47, 58, 73, 87) where they respond to microbiota-generated SCFAs to mediate physiological responses of the host (62). For example, Gpr41\(^{-/-}\) mice weigh less and gain weight at a slower rate compared with their wild-type littermates; furthermore, the differences in weight gain are dependent on gut microbiota (as illustrated by the lack of weight differences between genotypes in germ-free animals) (71). On the cellular level, other studies have shown that Gpr41 inhibits cell proliferation and induces apoptosis via the activation of p53 and MAPK (48, 88).

Gpr43 (free fatty acid receptor 2, Ffar2). Like Gpr41, Gpr43 was also found to be most responsive to propionate [EC\(_{50}\): 300 \(\mu\)M; (49)], although it can also be activated a number of other SCFAs, with acetate, propionate, and butyrate being the stron-
gest three ligands (49). Whereas Gpr41 couples to Gi, Gpr43 was found to couple to both Gi and Gq (7, 49). Gpr43 is expressed mainly in vasculature and immune cells including lymphocytes, neutrophils, monocytes, and peripheral blood mononuclear cells (PBMCs) (42, 42, 47, 73, 87). Functionally, Gpr43 has been shown to regulate inflammatory responses of the host in response to SCFAs produced by the gut microbiota (49, 54). SCFAs activate cytokines and chemokines both in cultured intestinal epithelial cells and in mice via the activation of Gpr43, as the response was absent in Gpr43−/− mice (46). In addition, Gpr43−/− mice have extensive dysregulation of inflammatory responses, showing excessive inflammation in models of colitis, arthritis, and asthma (54).

**SCFA Receptor: Gpr109a**

Gpr109a was initially identified as a receptor for niacin (75, 85) and subsequently was also found to respond to β-hydroxybutyrate as well as butyrate (70). Interestingly, this receptor does not respond to acetate or propionate, but it has an EC$_{50}$ for butyrate of ~1 mM (70). Gpr109a has been localized to epithelial cells in the colon (12), where its level of expression is suppressed in the absence of gut microbiota. Intriguingly, two studies published in early 2014 (20, 67) reported that activation of Gpr109a can suppress carcinogenesis.

**SCFA Receptor: Olfr78**

Recently, a number of “sensory” receptors (olfactory and taste receptors) have been shown to play important roles in a variety of tissues and physiological processes (17, 21, 29, 31, 32, 36, 64, 69). One such receptor is olfactory receptor 78 (Olfr78), which was an orphan receptor before a ligand screen identified it as a receptor for SCFAs (58). Olfr78 responds to acetate and propionate (EC$_{50}$ 2.35 mM and 920 μM, respectively), but it does not respond to butyrate (in contrast, Gpr109a responds only to butyrate). Importantly, the human ortholog of this receptor was independently found to also respond to SCFAs (92), and this finding has since been confirmed (58). These data, then, suggest that the function of this receptor is likely to be conserved by evolution.

The same study that deorphanized Olfr78 also reported that Olfr78, Gpr41, and Gpr43 localize to blood vessels, and that Olfr78 localizes to a specialized renal vessel (afferent arteriole) where renin is stored and secreted (58). Subsequently, it was demonstrated that both Olfr78 and Gpr41 play roles in sensing gut microbiota-derived SCFAs to modulate blood pressure.

**SCFA EFFECTS ON CELL BIOLOGY**

SCFAs have been shown to have effects on several aspects of cell biology, including histone acetylation, cell proliferation, and apoptosis. In this portion of the review, we will cover what is currently understood regarding these effects and the mechanisms underlying them.

**Modulation of Histone Acetylation by SCFAs**

Gene expression is regulated by modulation of histone acetylation by histone acetyl transferases (HATs) and histone deacetylases (HDACs) (53, 63). Addition of acetyl side chains to lysine residues on histones allows activation of transcription from specific regions of the genome. SCFA metabolites produced by microbiota inhibit histone deacetylase activity in cells thereby modulating gene expression in target cells (16, 35, 55, 72, 79). Butyrate is the most potent SCFA inhibitor of histone deacetylases, achieving ~80% in vitro inhibition of calf thymus HDAC1/2; propionate and pentanoate are the next most efficacious with a ~60% in vitro inhibition (11). Hyperacetylation of histones, stemming from SCFA-mediated inhibition of HDACs, changes chromatin structure. Thus, histone hyperacetylation alters the accessibility of transcription factors to specific genes, thereby causing SCFA-mediated alterations in gene expression (30).

Mechanistically, SCFAs function as noncompetitive inhibitors of HDACs, functioning effectively in vitro as well as in vivo with a $K_I$ ~60 μM for butyrate in vitro (11). Moreover, it has been shown that butyrate, propionate, and pyruvate (a 3 carbon intermediate of glycolysis, not a SCFA metabolite) specifically inhibit HDAC1 and HDAC3 (37, 74). Butyrate induces histone hyperacetylation in a wide spectrum of cell lines, both normal and cancerous (1, 44, 45, 51, 86). In cell-free extracts, addition of butyrate increases the half-life of acetyl groups added to histones by 150 times (11). However, butyrate does not affect the process of histone acetylation itself; therefore, the increase in half-life is due to inhibition of HDACs (11). HDAC inhibition is believed to be mediated directly by SCFAs, independent of GPCRs, based on the requirement for SLC5A8 for SCFA entry into colonocytes and subsequent HDAC inhibition (68); however, there is also evidence from another group that Gpr41 may help mediate SCFA effects on HDAC (86). 3-Hydroxy butyrate (β-hydroxybutyrate), a structurally similar ketone intermediate endogenously produced during starvation and diabetes, inhibits class I HDAC in HEK293T cells (65).

In addition to the gut microbiome, constituents of the oral microbiome produce SCFA metabolites that have similar effects on HDAC1/2 (90). In contrast to the gut where commensal symbiotic microbiota produce SCFAs, in the oral cavity, periodontal pathogens produce SCFAs that downregulate silent information regulator-1 (SIRT1) and two histone N-lysine methyltransferases (HMTs), EZH1 and SUV39H1, enabling Kaposi sarcoma herpesvirus lytic replication in cell lines and in patients with periodontal disease (90). Saliva of patients with periodontal disease has significantly higher concentrations of butyrate, isobutyrate, and propionate compared with healthy individuals (90).

**Cell Proliferation and Apoptosis**

SCFAs, especially butyrate, exhibit strong antitumorigenic properties, inhibit cell proliferation, and induce differentiation and apoptosis in a variety of cell lines, including human colorectal cancer cell lines HCT-116 and HT-29 (1, 3, 18, 24, 44, 48, 55, 72, 74, 86). In fact, SCFAs have been shown to confer protection from the development of colorectal cancer (3, 18, 23, 55, 72, 79). Propionate and butyrate have been shown to significantly inhibit cell proliferation, whereas acetate does not significantly affect cell proliferation (3, 44, 66). In addition, both propionate and butyrate have been shown to induce apoptosis in vitro (1, 9, 23). It has been hypothesized that inhibition of cell proliferation by butyrate is a direct consequence of HDAC inhibition (86).
Arrest of cell cycle following butyrate treatment occurs by both p53-dependent and -independent mechanisms, whereas apoptosis is initiated by a change in chromosomal structure leading to mitochondrial activation of caspase-3 (55). p53-dependent apoptosis occurs via Gpr41 signaling, as expression of Gpr41 in H9c2 cells increases accumulation of phosphorylated p53 in the nucleus, which in turn induces Bax expression, initiating apoptosis following hypoxia (48). Butyrate also enhances osteogenic differentiation while inhibiting adipogenic differentiation from model adult multipotent mesenchymal stem cells via HDAC inhibition and upregulation of ERK phosphorylation (9).

Mechanism of Butyrate Action

Comparative analysis of a butyrate-treated human colorectal carcinoma cell line, HCT-116, and its butyrate-insensitive derivative, HCT-116BR, revealed differential expression of genes involved in a wide variety of cellular functions, nearly half of the genes being involved in transcription, translation, and protein folding (23). It was found that butyrate downregulates key proteins associated with the actin cytoskeleton in both HT-29 and HCT-116 cells (23, 24). One of the genes downregulated by butyrate in HT-29 cells was cortactin, which is a key player in actin remodeling. Induction of apoptosis in butyrate-treated HT-29 cells is hypothesized to be caused by cortactin downregulation, which causes actin cytoskeletal remodeling (23). Furthermore, genes associated with inflammation, angiogenesis, immunoregulation, and structural proteins were differentially regulated by butyrate in HT-29 and HCT-116 cells. In addition, butyrate induces a cellular stress response in addition to endoplasmic reticulum (ER) and mitochondrial stress. ER chaperones heat shock protein A5 (HSPA5), endoplasmic reticulum protein 29 (ERP29), and protein disulfide isomerase family A member 3 (PDIA3) were differentially regulated upon butyrate treatment in HCT-116 cells (23, 24). Butyrate does not have any effect on total p38 levels in a cell; it rather alters phosphorylation of p38. Treatment of cells with butyrate downregulates HSP27 expression in addition to phosphorylation of HSP27 at Ser-15, -78, and -82 independent of p38 signaling (23, 88, 89). It is important to note that this study (23) was focused on the effect of butyrate on colorectal carcinoma progression, but did not carry out any analysis of the effects of other SCFAs (i.e., propionate or acetate). Hence, it is unknown whether propionate or acetate would have any similar effects on the genes differentially regulated by butyrate.

Physiological Consequences of SCFAs in the Whole Animal

Although this review has concentrated on the cell biological effects of SCFAs, it is important to remember that SCFA signaling has important physiological effects as well. For example, in whole animal studies, butyrate and valproate signaling has important physiological effects as well. For example, in whole animal studies, butyrate and valproate signaling has important physiological effects as well. For example, in whole animal studies, butyrate and valproate signaling has important physiological effects as well. For example, in whole animal studies, butyrate and valproate signaling has important physiological effects as well. For example, in whole animal studies, butyrate and valproate signaling has important physiological effects as well. For example, in whole animal studies, butyrate and valproate signaling has important physiological effects as well. For example, in whole animal studies, butyrate and valproate signaling has important physiological effects as well. 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CONCLUSION

Metabolites produced by gut microbiota modulate a variety of physiological pathways in the host (13, 19, 38, 49, 54, 58, 62, 80, 81, 84). One of the most well-studied modes of microbe-to-host communication is via SCFAs, which are produced by the breakdown of dietary fiber in the colon by microbiota. The highest concentrations of SCFAs are in the colon following a meal; from there, they are transported into the bloodstream where they can act via GPCRs—Gpr41, Gpr43, Olfr78, and Gpr109a. However, SCFAs can also act independent of GPCRs to modulate histone acetylation and cell proliferation. In the future, it will be necessary to understand the interaction between the physiological effects of SCFAs, and the cell biological changes that SCFAs induce. This is an extremely promising area of research; for example, a better understanding of the role of SCFAs in cell cycle inhibition may be therapeutically useful, as SCFAs are known to be antitumorogenic. In the future, it is especially exciting to consider that modulating metabolite production by the gut microbiota may be a useful tool to obtain beneficial effects on host physiology.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

N.N. and J.L.P. drafted manuscript; N.N. and J.L.P. edited and revised manuscript; J.L.P. approved final version of manuscript.

REFERENCES


