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Role of Short Chain Fatty Acid Receptors in Intestinal Physiology and Pathophysiology

Medha Priyadarshini[1], **Kumar U. Kotlo**[1], **Pradeep K. Dudeja**[2,3], and **Brian T. Layden**[*,1,3]

[1]Division of Endocrinology, Diabetes and Metabolism, Department of Medicine, University of Illinois at Chicago, Illinois, USA

[2]Division of Gastroenterology and Hepatology, Department of Medicine, University of Illinois at Chicago, Illinois, USA

[3]Jesse Brown Veterans Affairs Medical Center, Chicago, Illinois, USA

Abstract

Nutrient sensing is a mechanism for organisms to sense their environment. In larger animals, including humans, the intestinal tract is a major site of nutrient sensing for the body, not surprisingly, as this is the central location where nutrients are absorbed. In the gut, bacterial fermentation results in generation of short chain fatty acids (SCFAs), a class of nutrients, which are sensed by specific membrane bound receptors, FFA2, FFA3, GPR109a, and Olfr78. These receptors are expressed uniquely throughout the gut and signal through distinct mechanisms. To date, the emerging data suggests a role of these receptors in normal and pathological conditions. The overall function of these receptors is to regulate aspects of intestinal motility, hormone secretion, maintenance of the epithelial barrier, and immune cell function. Besides in intestinal health, a prominent role of these receptors has emerged in modulation of inflammatory and immune responses during pathological conditions. Moreover, these receptors are being revealed to interact with the gut microbiota. This review article updates the current body of knowledge on SCFA sensing receptors in the gut and their roles in intestinal health and disease as well as in whole body energy homeostasis.

Introduction

> “Man is what he eats”
>
> —(Ludwig Feuerbach, 1826)

As an interpretation of Feuerbach’s words, a profound association of diet with health of the body exists. The intestine with its specialized architecture functions to digest and absorb nutrients (211). It also sends messages to the body to relay information on the metabolic processes occurring in the gut. This role of the intestine is facilitated by an assortment of molecular sensors that provide feedback about the environment to the host (202). Undoubtedly, some of these molecular sensors have been shown to be specific to nutrients (202). Nutrients, including carbohydrates, amino acids, and fatty acids, which are essential

*Correspondence to blayde1@uic.edu.

not only for growth and sustenance, also interact with metabolic and immune systems regulating multiple aspects of health of the host (80). The nutrients interact with these systems through trans-membrane or intracellular molecules, which then transduce “the detection event” to specific cellular responses (152).

While sensing the environment is an important survival technique for single cell organisms, which allows the cell to process cues for self-protection and nutrient availability, this mechanism has continued into more complex organisms including mammals. Most mammalian cells are now known to employ multiple nutrient sensing mechanisms for proper cellular function (106). One type of nutrient sensors, the nutrient sensing G-protein coupled receptor(s) (GPCR), are a versatile class with regards to ligand diversity, ability to act via varied signaling pathways, and widespread tissue expression (152). Over the past 20 years, receptors for almost every nutrient have been revealed (Table 1) (25). The discovery of these GPCRs has dramatically changed our understanding of how the body responds to nutrients, and has heralded great efforts, as these receptors are excellent drug targets (25). An understanding of their signaling mechanisms through G-proteins restricted to cell membranes, and more recently described, their intracellular endosomal signaling has revealed insights into not only how cells sense nutrients (or other molecules like hormones) but also into the extensive roles these receptors play in regulating cell physiology.

While these receptors are expressed broadly, as expected, many of these nutrient-sensing GPCRs are present in intestinal cells (217), as this is the main site for processing and absorption of nutrients. Additionally, these gut nutrient sensing GPCRs have been shown to contribute to gut hormone secretion (129). Among the various nutrient-sensing GPCRs of the intestine, one class responding to free fatty acids (FFA) is the FFA sensing receptor (FFAR) class. FFA ligands for these receptors range in size, from short, medium to long carbon chain length, ranging from C = 2 to 6, C = 6 to 12, and C > 12, respectively (Table 1). This FFAR class is currently comprised of four members, receptors binding long chain fatty acids, free fatty acid receptor 1, FFA1 (previously GPR40) and FFA4 (previously GPR120) and receptors binding short chain fatty acids, FFA2 (previously GPR43) and FFA3 (previously GPR41) (Table 1) (3). Another GPCR, GPR84 reported to bind medium chain fatty acids (carbon chain length ranging from C = 6 to C = 12) lacks sufficient data to be considered as FFA5 and is still classified as orphan (213). The long chain fatty acids like palmitoleic and linoleic acids, which activate FFA1 and FFA4, are either diet derived or produced endogenously during fatty acid metabolism (205). While sensing long chain fatty acids, both of these long chain fatty acid receptors, can enhance insulin secretion by activity on pancreatic beta (β) cell (FFA1) and influence incretin secretion from enteroendocrine cells (FFA1 and FFA4) (61, 88, 113), and thus, modulate whole body energy utilization.

The short chain fatty acids (SCFAs), the ligands of FFA2 and FFA3, are distinct given the nature of their origin. SCFAs are largely thought to be produced by the fermentation of ingested complex carbohydrates by intestinal bacteria (98, 110). The SCFAs are sensed by these receptors, and through this interaction, modulate a variety of physiological and hormonal processes that contribute to whole body energy sensing (33, 98, 152). Consequently, SCFA-cognate receptor signaling presents a novel intestinal (gut) relationship to the intestinal function and to the rest of the body, primarily through hormonal secretion.

In addition to FFA2 and FFA3, other GPCRs that can be modulated by SCFAs have been discovered namely, Olfr78 and GPR109a. While not classified as part of the FFAR family of GPCRs, these GPCRs are regulated by SCFAs and/or closely related SCFA intermediates, as we describe below. In this article, we present the current knowledge on the role of SCFA receptors with an emphasis on their function in intestinal physiology and pathophysiology, and will also enumerate on their interrelationship with gut bacteria and the therapeutic relevance in intestinal metabolic and inflammatory disorders.

Diverse Signaling Mechanisms of GPCRs

GPCRs are seven transmembrane spanning proteins, and are the largest receptor class in the human genome. GPCRs mediate their individual signaling characteristics through multiple mechanisms, where the most well characterized mode is via Guanine nucleotide binding proteins (G-proteins) (99). G-proteins are heterotrimeric proteins, containing alpha, beta, and gamma subunits (162). In humans, four primary G-protein alpha subunit classes ($G\alpha_s$, $G\alpha_{i/o}$, $G\alpha_{q/11}$, and $G\alpha_{12/13}$) are predominant, where each alpha subclass influences important and distinct intracellular pathways (Fig. 1) (162). $G\beta\gamma$ proteins can also mediate their own signaling pathways, and this subunit remains a complex, and does not dissociate. Overall, there are 5 distinct beta and 12 distinct gamma subunits that are expressed in humans (90).

Upon GPCR activation, by their specific endogenous ligand or pharmacologic agonist, a conformational change occurs across the GPCR, leading to internal GPCR domain changes in the GPCR and the G-protein complex structural interface (138). This process allows the G-protein complex to be activated, where the GDP on $G\alpha$ is replaced by GTP, resulting in $G\alpha$ and $G\beta\gamma$ free to signal the external “ligand” message to the internal mileu (138). While this classical GPCR signaling mechanism has been well validated and characterized, a multitude of mechanisms have been shown to affect each step of this process. A few of these mechanisms are depicted in Figure 2. Along with the availability of a ligand to activate the GPCR, the amount of the plasma membrane receptor available for ligand-induced activation is regulated through desensitization and resensitization processes (14), which are essential to prevent overstimulation of the GPCR and for the recovery of a functional receptor, respectively. Next, the step where GPCR activity is regulated is the GTP hydrolysis rate of the $G\alpha$ proteins (Fig. 2A). Within the cell, proteins that modulate the intrinsic time of $G\alpha$ activation influence duration of ligand effect. These special proteins called “regulators of G-protein signaling” (RGS proteins) aid $G\alpha$ proteins in this function and enhance their GTP hydrolysis potential. GPCR activity is also regulated through the G-protein receptor kinases or GRKs (Fig. 2B). GRKs can phosphorylate specific residues in the cytoplasmic domains of ligand-activated GPCRs allowing dissociation of G-protein subunits from the receptor (112). β-arrestin is another multifunctional protein that acts in concert with GRK regulating desensitization of most receptors (Fig. 2C). Following GRK mediated phosphorylation, β-arrestin binds ligand-activated GPCRs and inhibits G-protein coupling (144). β-arrestin additionally can function as a scaffold for various adaptor proteins like clathrin and promote receptor internalization (endocytosis). The GPCR-arrestin endosome can also have multiple fates. First, GPCR-arrestin complex can direct assembly of a “signalosome” with recruitment of different kinases e.g. mitogen activated kinases like ERK1/2, and c-Jun N

terminal kinases (JNK) allowing G-protein-independent signaling by the receptor (Fig. 2D) (81, 112, 179). Second, internalized receptor can either be recycled back to the plasma membrane or third, it can be degraded (Fig. 2E-F). Moreover, the amount of ligand can modulate the response of the GPCRs where higher concentration of the ligand often leads to a greater response (24). Ligands can also show selectivity for β-arrestin-dependent or G-protein-dependent signaling and this mechanism is called as biased agonism (144) (Fig. 2G). Additionally, transcriptional regulation of receptor expression is another mechanism for regulation of GPCR levels (Fig. 2H). And lastly, GPCR dimerization processes occur between the similar and dissimilar GPCRs (homo or hetero-dimers) to modulate the activation of the GPCR and its signaling capabilities (50) (Fig. 2I).

Among the SCFA receptors, data exists beyond the classic G-protein signaling for FFA2 and GPR109a where β-arrestin-dependent mechanisms have been described. Human FFA2 has been shown to employ β-arrestin-dependent signaling for transcriptional regulation of proinflammatory cytokine expression *in vitro* (102). GPR109a when activated by nicotinic acid (and not SCFAs, *an example of biased agonism)* has been shown to recruit ß-arrestin 1 and mediate cutaneous flushing effect associated with nicotinic acid consumption (212). For FFA2, ligand bias extends to selective coupling of Gα proteins. Synthetic ligands at FFA2 can elicit either $G\alpha_{i/o}$ or $G\alpha_{q/11}$ signaling (27) and also physiological conditions like high-fat diet consumption can influence G-protein coupling bias of the receptor itself in favor of $G\alpha_{q/11}$ over $G\alpha_{i/o}$ (120). Furthermore, physiological ligand concentrations possibly affect the SCFA receptors activity (and probably expression) in yet another way. Highest concentration of their ligands (SCFAs) is present in the intestine where butyrate is consumed as energy source and remaining SCFA pool is drained into the portal vein (40). Liver metabolizes propionate, leaving acetate as the SCFA with highest systemic concentration (40). Accordingly, prominent physiological effects of these receptors are noted in the intestine, as discussed in the following text.

In summary, it is clear that GPCR signaling is complex and new modes of signaling are being discovered (for greater detail on GPCR signaling mechanisms and regulation, see Further Reading list). In this overview article, we focus on how SCFA receptors (see Table 2) signal via Gα (and through other pathways, if known) and influence different normal and pathological cellular processes in the gut.

The Short Chain Fatty Acid Receptors

FFA2 and FFA3

Discovery: The discovery of the first short chain fatty acid receptors (SCFA-Rs) and their ligands occurred serendipitously. During the search for human galanin receptor subtypes, a cluster of four intron-less genes, at the time named as GPR40, GPR41, GPR42, and GPR43, was identified downstream of CD22 gene on chromosome 19q13.1 (166). Based on their resemblance with members of a GPCR class (in particular, the class A receptors), these were classified as orphan GPCRs, where this term orphan refers to the lack of known ligand responsible for the particular GPCR [for greater detail on GPCR classes see (145)]. Gene cluster of these FFARs in mice exists on chromosome 7 but devoid of GPR42 which is a feature observed only in the primate FFAR gene cluster (155). A few years later, the first

rodent orthologs of FFA2 were cloned from leukocytes and FFA3 from lungs (29, 171). Since then, orthologs from various species have been cloned and characterized (121). Because these receptors (FFA2 and FFA3) are genetically close and were discovered at the same time, we discuss them here together.

Following the discovery of these genes, screening of ligands active at these receptors using recombinant cell systems, reporter gene assays, and Ca^{2+}mobilization/cAMP accumulation assays was done (97, 130). In 2003, multiple groups reported SCFAs to be ligands for FFA2 and FFA3 (32, 100, 130). It was a chance observation that only the compounds with acetate as a counter ion could elicit FFA2 activity. Later acetate alone, salts of acetate and other carboxylate ions up to six carbons in length were revealed to activate both receptors, albeit with different potencies (32, 100, 130). GPR42, which shared 98% homology with FFA3 (166), however, was not activated by any of the SCFAs and was classified as a pseudogene (32). Potentially of importance, it was recently suggested that GPR42 expressed heterologously in rat sympathetic neurons is functional exhibiting lower potency but similar efficacy as FFA3 for propionate (155). The discrepant observations of functional activity of GPR42 in studies of Brown et al. (32) and Puhl et al. (155) may have arisen from use of different assays monitoring the activity. However, if GPR42 truly represents a functionally important gene remains to be confirmed.

Pharmacology: Owing to 43% amino acid sequence identity, it is not surprising that FFA2 and FFA3 also share overlapping ligand selectivity profiles. The consensus of all reports is that the shorter the carbon chain length of the SCFA, the higher the potency at FFA2 and vice versa for FFA3. Accordingly, acetate (C2) and propionate (C3) show highest and equal potency at FFA2 followed by butyrate (C4) (Table 3) (32, 76, 130, 183). Formate (C1) and pentanoate (C5) can also activate FFA2 but to a lesser extent while hexanoate (C6) shows no activity with FFA2 (28, 130). At FFA3, C3, C4, and C5 show highest and equal potency, C2 and C6 can also activate the receptor but to a lesser extent (Table 3) (124). SCFAs with substitution of branched chain alkyl groups are more potent at FFA3 (168). Modifications of SCFA alkyl chain with unsaturated double or triple bond favor FFA2 over FFA3 (168).

With regards to SCFA potency, an important distinction between human and mouse orthologs of the receptors has been revealed. First, C2 is 20-fold more selective at human FFA2 than at human FFA3 while C2 is equipotent at both the mouse orthologs (168). Next, C3 is 12-fold more selective at mouse FFA3 while exhibiting similar potency at human orthologs (168). Ionic interactions between positively charged amino acid residues and carboxylate group of SCFAs are essential to ligand binding and receptor function (183), and it has been shown that the molecular basis of specific ligand selectivity described above lies in the differences in core amino acids constituting the binding pockets of these receptors (183). These observations will be important as pharmacologic molecules have been developed and some of which are being tested in preclinical studies.

Expression: Both FFA2 and FFA3 are highly expressed in the intestine (Fig. 3). This high expression at the natural site of their ligand production may have evolved as a part of mutualistic association between the gut microbiota and the host. Dass et al. (42) and Karaki et al. (84) were the first reports describing expression of these GPCRs throughout the

gastrointestinal (GI) tract. First, the highest expression for FFA2 was observed in the rat colon (42), and its expression was also reported in enteroen-docrine cells expressing (peptide YY) PYY and in mucosal mast cells that express serotonin (84), an observation supported by the same group in human large intestine (85). Of note, enteroendocrine cells are specialized cells of the GI system that release gastrointestinal hormones into blood stream producing an array of systemic metabolic and immunologic effects. There are several types of intestinal endocrine cells including I cells, which secrete cholecystokinin (CCK), K cells that secrete gastric inhibitory peptide (GIP), L cells that secrete glucagon-like peptide 1 (GLP-1), GLP-2 and PYY, and enterochromaffin cells that secrete serotonin. FFA3 has also been observed to be expressed in intestinal tissue, where it was first reported that FFA3 was expressed in the human colon(enterocytes and enteroendocrine cells) but to a lesser extent than FFA2 (197). Immunohistochemistry studies have shown that FFA3 is expressed in the apical cytoplasm of enterocytes, enteroendocrine cells, and several lamina propria cells but not in 5-HT (5-hydroxytryptamine) positive enterochromaf-fin cells of the human colon. All FFA3 positive enteroen-docrine cells were reported to be immunoreactive for PYY producing cells but not vice versa. Interestingly FFA3 was not co-localized in FFA2 immunoreactive enteroendocrine cells (197). In mice, Samuel et al. (165) showed that FFA3 is highly expressed in small intestine especially in ileum. Further studies from Sykaras et al. in mice reported the expression of FFA3 and FFA2 transcripts in duodenal I-cells (188).

Other than the gut, FFA2 expression is localized to many immune cell types including regulatory T cells (6, 37, 84, 100, 181), bone marrow, and adipose tissue (56, 70, 105, 116). Immune cell expression of FFA2 has been observed to change in response to cell maturation and inflammation (6, 116, 171, 174). For example, in human monocytes, treatment with lipopolysaccharide, tumor necrosis factor (TNF) or granulocyte macrophage colony stimulating factor (GM-CSF) can upregulate FFA2 expression via actions of transcription factors like nuclear factor kB (NFkB) and x-box binding protein-1 (XBP-1) on FFA2 promoter (Fig. 2H) (6, 171, 174). Modulation of FFA2 expression in immune cells consequent to inflammation may indicate the crucial role FFA2 plays in regulating number and function of these cell types in mitigating adverse inflammatory reactions (59, 115).

FFA2 has also been observed to be expressed in gastric ghrelin cells (49) and both FFA2 and FFA3 in gastric brush cells, islet α and β cells, renal cortical epithelial cells and spleen (13, 47, 56, 89, 95, 100, 120, 152, 192, 207). The expression of FFA3 in β cells is regulated in a unique fashion. Its expression is mediated via internal ribosome entry site-dependent translation of bicistronic mRNA encoding FFA3 and GPR40 (13). Additionally, FFA3 expression has been noted in sympathetic and enteric neurons and autonomic ganglia (43, 131, 132), certain immune cells like dendritic cells and monocytes (100), and spleen (Fig. 3) (56, 95, 100). Hence, FFA3 seems to be more widely expressed as compared to FFA2. Its expression in adipose tissue, however, is debated. Initial reports (32, 100, 223) suggesting expression of FFA3 in adipose tissue or cell lines were later refuted when no FFA3 was detected in different adipose depots or confluent and differentiated adipocytes (68, 228). More recent reports have again reinforced its presence in adipose tissue. Using 3T3-L1 adipocyte cell line, these reports have projected a role of adipose expressed FFA3 in insulin signaling and lipolysis (64, 136). While these reports await validation of FFA3 protein level in adipose tissue, it has been identified that its expression in this tissue can be induced under

certain conditions such as the lack of FFA2 (in FFA2 knockout mice), dietary SCFA supplementation, or the stage of adipose tissue development in some mammals (23, 105, 108).

Majority of the data on expression of FFA2 and FFA3 (and other nutrient sensing GPCRs in general) has been generated through transcriptomic analyses and there may be uncertainty with detection of false positives (use of primers spanning introns and inappropriate controls, low transcript levels in certain tissues). Moreover, in absence of any reliable antibodies (191, 207, 209) transcript level analysis is the only option currently available for determination of expression of these receptors except for the use of reporter proteins expressed under control of their promoters. An elegant method performed by Nohr et al. (131, 132) used transgenic reporter mice expressing monomeric red fluorescent protein (mRFP) under the control of FFA2 and FFA3 promoters to evaluate their expression patterns in GI tract. Their findings indicated that in the gastric mucosa, FFA3-mRFP is expressed in gastrin and ghrelin-positive cells and in the small intestine, FFA3-mRFP is expressed mainly in the CCK-secretin-GIP-GLP-1-PYY-neurotensin lineage of enteroendocrine cells. Although earlier studies showed expression of FFA2 in GLP-1 positive enteroendocrine cells, utilizing Q-PCR (quantitative polymerase chain reaction), FACS (fluorescence activated cell sorting) analysis and immunohistochemistry, Nohr et al. (131, 132) demonstrated that FFA2 is strongly expressed in enteric leukocytes and rather weakly expressed and only in a fraction of the enteroendocrine cells. Use of similar model for assessment of FFA2 and FFA3 expression in other tissues like adipose will be important. Such models may also be helpful in elucidating mechanisms governing membrane expression and non-G-protein signaling of these receptors.

Signaling: Unique G-protein alpha signaling characteristics of FFA2 and FFA3 lead to distinct downstream effects (Fig. 4). FFA2 is reported to be capable of dual signaling via $G\alpha_{q/11}$ and $G\alpha_{i/o}$ pathways; whereas, FFA3 signals exclusively by $G\alpha_{i/o}$. Because of these G-protein-coupling patterns, FFA3 activation predominantly has an inhibitory tone which is associated with inhibition of adenylyl cyclase activity and reduction in intracellular cyclic adenosine monophosphate (cAMP) levels (Fig. 1) (32, 76, 100, 152, 183). Propionate induced activation of FFA3 in islets, for example, inhibits insulin secretion in pertussis toxin (a GPCR-$G\alpha_{i/o}$ interaction inhibitor) sensitive manner (150). Interestingly though, $G\alpha_{i/o}$ coupled FFA3 can also induce excitatory responses like sympathetic activation; however, this is via $G\beta\gamma$-PLC-MAPK pathway (Figs. 1 & 4) (93). As of yet, there are no findings demonstrating the recruitment of GRK-arrestin by ligand activated FFA3 (75, 93) except for the latest report, where ligand activation of heterologously expressed human FFA3 recruited ß-arrestin 2(8).

FFA2 is more versatile than FFA3, as it signals by multiple G-protein signaling pathways. More exactly, it can signal via $G\alpha_{q/11}$, $G\alpha_{i/o}$, and $G\alpha_{12/13}$, and can, therefore, inhibit or activate cellular responses (32, 76, 100, 130, 183) (Fig. 4). For instance, FFA2 activation can enhance insulin secretion via $G\alpha_{q/11}$ (120, 151) and inhibit ghrelin secretion through $G\alpha_{i/o}$ pathway (49). Similar to FFA3, FFA2 can also signal through $G\alpha_{i/o}$. In adipocytes, acetate and agonists at FFA2 inhibit insulin signaling in pertussis toxin and Gallein (which is a $G\beta\gamma$ blocker) sensitive manner (94). Activated FFA2 can also recruit arrestin (73, 74, 102, 226).

Ligand-activated FFA2 has been shown to recruit ß-arrestin 2, leading to internalized FFA2, and signal dephosphorylation (inactivation) of NFkB (102). This effect has been associated with reduced expression of proinflammatory cytokines in *in vitro* studies (102).

Ability to evoke different signaling pathways by ligands (referred to as ligand bias) provides another level of dexterity in FFA2 signaling. More precisely, ligand bias refers to the unique ability of a compound to favor one signaling pathway over the other. Several synthetic ligands at FFA2 have been described to selectively elicit $G\alpha_{q/11}$, $G\alpha_{i/o}$, or β-arrestin pathway (28). One of the first potent activators of FFA2, 4-chloro-α-(1-methylethyl)-N-2-thiazolylbenzeneacetamide (4-CMTB) was originally reported to activate $G\alpha_{q/11}$, $G\alpha_{i/o}$ as well as β-arrestin pathways (103, 180, 216). In our laboratory, glucose-stimulated insulin secretion (GSIS) in studies utilizing islets from FFA2 knockout (KO) and wild-type (WT) mice suggested that this compound showed bias for $G\alpha_{i/o}$ over $G\alpha_{q/11}$ (151). Moreover, in *in vitro* Ca^{2+} mobilization assays (a substitute technique for studying $G\alpha_{q/11}$ response) in mouse β cell line, we did not observe any activity of this compound at FFA2 (151). Though these data suggest biased agonism by 4-CMTB, this observation, however, requires further investigation.

Previously unknown, FFA2-FFA3 heteromerization shown by Ang et al. recently in both *in vivo* and *in vitro* settings has further added complexity to signaling mechanisms of these receptors (8). Heteromers of human FFA2-FFA3 exhibit distinct signaling with: (1) significant elevation in Ca^{2+}mobilization above what was observed for FFA2 alone; (2) marked enhancement in β-arrestin 2 recruitment and; (3) loss of $G\alpha_{i/o}$ signaling (Fig. 2I) (8). A limitation of these observations is the narrow tissue range screened for this heteromer where only macrophages and monocytes exhibited heteromers but not colonic epithelial cells. Physiological significance of FFA2-FFA3 heteromer and its presence in other tissues still needs clarification.

SCFAs, the natural ligands of these receptors, show species bias at these receptors owing to differences in their ligand binding pockets and their constitutive signaling properties. For example, human FFA2 exhibits high affinity for acetate and also has a high level of constitutive activity, whereas human FFA3 does not exhibit these properties. Moreover, differences in these characteristics exist between species (human vs. mouse isoforms) for the same receptor (73). How this relates to their biological function requires more investigation. For example, acetate enhances GSIS in mouse islets via $G\alpha_{q/11}$ pathway but has no net effect on GSIS in the human islets where it evokes both $G\alpha_{q/11}$ and $G\alpha_{i/o}$ pathways (120, 151). Therefore, use of SCFAs and synthetic ligands at these receptors in *in vivo* settings is challenging, as a means to understand receptor biology. Concomitant use of receptor knockout and overexpression mouse/cell models along with the models expressing the wild-type receptors has allowed partial segregation of tissue specific effects of these receptors (56, 94, 151, 191, 192, 207). As discussed in subsequent sections, FFA2 and FFA3 appear to have roles in numerous health conditions. Hence, synthesis and characterization of specific and selective agonists for these receptors is important for their use in understanding each of their biological effects.

Role of FFA2 and FFA3 in the gut physiology

SCFAs are generated in the colon as end products of bacterial fermentation where the primary ones are acetate, propionate, and butyrate (98). In colon, SCFAs play critical roles as predominant source of metabolic energy, maintenance of epithelial integrity, fluid absorption and have important anti-inflammatory and anti-cancer effects (66, 87, 156, 178, 187, 193, 194). These SCFAs specifically also affect important cellular processes such as proliferation, differentiation, apoptosis, hormonal secretion, and immune responses in the gut, and in part via activation of FFA2 and FFA3 (5, 31, 52, 92, 111, 116, 154, 165, 181, 196), as outlined later.

Role of FFA2 and FFA3 in epithelial barrier function and intestinal inflammation—SCFAs have long been known to exert beneficial effects against intestinal inflammation and protecting intestinal epithelial integrity (66, 87, 187). Recent studies have shown that these protective effects of SCFAs are, in part, through their receptors, FFA2 and FFA3. Loss of these receptors in various experimental models of intestinal inflammatory disorders like inflammatory bowel disease (IBD) has been associated with dysregulated inflammatory responses to different immunologic challenges (92, 111).

The physical barrier of gut epithelium offers first line of defense against injury or infection and optimal gut permeability is associated with development of protective immune response (204). In fact, modest disruption of the epithelial barrier is important to mount proper inflammatory response against pathogenic microbes and antigens (26). Because of their expression patterns, a role for FFA2 and FFA3 in regulation of epithelial barrier function has been investigated. For example, challenging the gut with 2,4,6-trinitrobenzene sulfonic acid (TNBS) increases gut permeability (214) and colonic neutrophil migration only in WT mice but not in mice deficient in FFA2 and FFA3 (92). Also, subsequent to TNBS administration, interleukin-6 (IL-6), IL-12, interferon γ (IFN-γ), and other inflammation-associated genes are induced in the colon of WT mice, but not in FFA2 and FFA3 KO mice (92).

In these above studies, the TNBS treatment led to early proinflammatory changes and set the stage for ousting the immunologic challenge over the long course. These effects were almost absent in FFA2 and FFA3 KO mice (26, 91). Owing to lack of such early inflammatory response, FFA2 KO and FFA3 KO mice show higher susceptibility to *C rodentium* infection (monitored for 4 weeks post insult), decreased pathogen clearance during the course of infection and reduced Th1 and Th17 responses (92), which were suggested to indicate that FFA2 and FFA3 are crucial regulators of gut permeability (92). FFA2 on dendritic cells can also promote intestinal IgA production, providing additional route of intestinal epithelium protection against pathogenic microbes (190, 220). Similarly, in acute as well as chronic DSS (dextran sodium sulfate) colitis model, which is independent of adaptive immune system, genetic knockout of FFA2 exacerbated the disease, through effects on neutrophil recruitment, cytokine release and gut integrity (111, 116, 117). Mediation of above effects by FFA2 and FFA3 signaling is possible through several mechanisms. SCFAs can induce anti-microbial peptide (AMP) production in gastrointestinal cell lines (167) while in islets this effect of SCFAs probably involves FFA2/3 (185). Islet AMP has immunomodulatory role and induces regulatory dendritic cells, T-cells, and macrophages (185). A similar role of

intestinal FFA2 and FFA3 is yet to be determined (79, 185). However, stimulated FFA2 on both gut epithelial cells and immune cells can affect production of immunomodulatory cytokines and regulatory T cells (Treg) and activation of inflammasome (92, 111, 116, 117, 133, 181).

Intestinal epithelial cells (IECs) secrete mucin, antibacterial peptides, and immune mediators that recruit or activate immune cells (143). Moreover, FFA2 and FFA3 expressed in IECs, may mediate some of the above effects. Bone marrow chimera studies have demonstrated a role of IEC-FFA2 in regulation of inflammation (92,111). For example, in DSS, TNBS, and infectious colitis models, FFA2 in nonhematopoietic cell population has emerged as a main player in promotion of gut homeostasis. Transfer of bone marrow from FFA2-KO (donor) to WT (recipient, ensuring absence of the receptor in myeloid cells) was protective against inflammation as compared to transfer of bone marrow from WT→KO. FFA2 in gut epithelia has also been shown to stimulate potassium ion efflux, hyperpolarization of membrane and activate NLRP3 inflammasome pathway via $G\alpha_{i/o}$ (111). All these processes attenuate colitis by stimulating the expression of IL-18 (111), a critical regulator of gut epithelial integrity (133). FFA3, unlike FFA2, seems to have a more restricted function in gut barrier preservation. FFA3 in the nonimmune cell population of the gut has been shown to confer protection in infectious colitis model mainly by enhancing expression of cytokines and chemokines through MEK-ERK (mitogen-activated protein/extracellular signal regulated kinase kinase) pathway (92). Protective effects of dietary manipulation on mucosal barrier in swine model of weaning associated diarrhea were conjectured to be mediated by enhanced levels of FFA3 in the gut (65). Similarly, protection observed with dietary manipulation in type 1 diabetic FFA2 KO mice is thought to be FFA3 driven (115). Though promising, these data need to be corroborated with loss of function studies in FFA3 knockout models.

Additionally, there is growing evidence that FFA2 modulates immune responses by regulating neutrophil chemotaxis, recruitment of inflammatory mediators and modulation of Treg development (92, 116, 117, 181,210). In both acute (2.5% DSS, 7 days) and chronic (0–6 days 4% DSS, 20–24 days 2%, and 35–39 days 4% DSS) colitis models, whole body FFA2 deficiency results in unabated inflammatory response (116), which includes increased neutrophil infiltration, concomitant increase in myeloperoxidase (MPO) activity with physical features of colitis, for example, reduced colon length (116). Acetate administration could reverse disease features in acute DSS model but only in the WT mice (116). Concordant with a “neutrophil-driven response,” WT mice with bone marrow reconstituted from FFA2 KO mice were not protected from DSS colitis suggesting that immune cell FFA2 is responsible for the phenotype observed in the KO mice (116). Kamp et al. also recently demonstrated that acetate-FFA2 ligand-receptor pair affects neutrophil migration following inflammatory stimulus (82). Of note, FFA2 is highly expressed in neutrophils (6, 32, 130). In light of these above studies, FFA2 appears to be crucial for regulating migratory behavior of neutrophils, and essential to mount anti-inflammatory response in acute as well as chronic inflammation conditions. Similar exacerbation of inflammatory response along with increased colonic levels of IL17 producing T helper cells (particularly CD44+IL17+ T cells) and cytokines has also been shown in TNBS colitis in FFA2 KO mice (116).

Another mechanism by which FFA2 can affect immune homeostasis is through the regulation of the number and function of Tregs, which are specialized T cells noted for immune-suppressive effects against autoantigens (self-antigens) (164). Interestingly, their expansion in the intestine is regulated by SCFAs (140, 181) and involves FFA2 signaling (181, 190). For example, in a study using T cell transfer colitis model, it was shown that genetic deficiency of the receptor could lead to insufficient number of colonic Tregs and reduced antiinflammatory IL-10 levels in intestinal inflammation (181), an effect not reverted by SCFA treatment. Thus, Treg deficiency in part could arise from a role of FFA2 in regulating colonic migration of extraintestinal Tregs (181). Influence of FFA2 signaling on Treg differentiation is, however, debated. Other studies have shown that SCFAs promote differentiation of gut $CD4^+$ T cells to $FOXP3^+$ Treg cells independent of the receptor possibly through histone deacetylase inhibition (9, 20, 57, 140). FFA2 may also affect Treg differentiation indirectly by regulating $CD103^+$ dendritic cells that are chief regulators of inducible mucosal Treg cells (190). Another possibility is that direct regulation of Tregs via FFA2 is tissue specific. For example, FFA2-dependent increase in Treg pool in type 1 diabetes model has been noted but only in peripheral tissues (115). FFA2, thus, has emerged as a key determinant of inflammatory response in intestine, affecting gut integrity, MPO activity, $CD44^+IL17^+$ T cells, $FOXP3^+$ Treg cells, and levels of proinflammatory cytokines in the colon tissue (92, 115, 116, 181).

Gut immune homeostasis as achieved by FFA2 and FFA3 signaling can have far-reaching effects (92, 204, 220). Not only SCFA-FFA2 primed gut immune cells can translocate to distant organs but also disrupted intestinal epithelial barrier results in leakage of pathogenic bacteria or their byproducts into systemic circulation (20, 204). For example, FFA2 KO mice were reported to have exacerbated immune response in asthma and arthritis (116) and WT mice showed protection against lung inflammation by FFA3-dependent regulation of production of macrophages and dendritic cells (203). The significance of these defensive roles of FFA2 and FFA3, however, has not been consistently observed (5). Sina et al. (175) and Kim et al. (92) reported contradictory findings as they observed opposing roles for protection against various immunologic insults in FFA2 KO compared to WT mice. The basis for these controversies is not entirely clear and may involve different experimental design/protocols in different studies. While Maslowski et al. suggested that bone marrow-derived cells contribute to the colitis phenotype (116), Kim et al. reported opposite findings by implicating suppression of FFA2-dependent activation of extracellular signal-regulated kinase 1/2 and p38 mitogen-activated protein kinase signaling in colonic epithelial cells accountable for the exacerbation of colitis (92). In contrast, Sina et al. (175) demonstrated that activation of p38 mitogen-activated protein kinase by FFA2 in polymorphonuclear leukocytes plays a key role in the pathogenesis of colitis. Interestingly, FFA2-mediated activation of the inflammasome is shown to be protective for gut homeostasis (111) but contributes to development of gouty arthritis (208). Nevertheless, immune-modulatory role of these receptors is likely a balance of pro- or anti-inflammation driven by multiple factors like disease course, gut microbes, and nutritional status (Fig. 5). Furthermore, differences in precise determination of expression of FFA2 in immune cells (191) and possible transcriptional regulation by SCFAs (108), physiological conditions (56, 116, 120, 151), and stress-induced inflammatory mediators like TNFα (6, 86) may be contributing to these

divergent effects. Further detailed studies in intestine-specific or cell-specific deletion of these receptors are needed to pinpoint the exact role of these receptors in IBD.

Role of FFA2 and FFA3 in colon cancer—Tumorigenesis and inflammation have interrelated features (71, 186). As such, a role of FFA2 in intestinal cancer may exist, similar to its reported role in mediation of inflammatory responses (111, 177). First of all, FFA2 is consistently down-regulated in cancer tissues and cell lines. For example, its expression was markedly reduced or completely absent in colorectal adenocarcinoma tissues compared to normal colon tissue in humans (177, 195). Going beyond expression changes, various reports have shown that FFA2 activity has antiproliferative effects on cancer cells (21, 22). Tang et al., (195) showed that restoration of FFA2 expression in colon cancer cell lines induced apoptosis and inhibited cell proliferation by arresting cells in G0/G1 phase, upregulating p21, and decreasing the levels of cyclin D3- and cyclin-dependent kinases (CDKs) 1 and 2. Indeed, FFA2 deficiency results in increased number and size of polyps and tumors in the colon of different cancer models, like $Apc^{Min/+}$ mice and mice cotreated with azoxymethane and DSS (111, 139, 177). Dysregulated inflammatory response with enhanced neutrophil migration and increased expression of proinflammatory markers such as IL-1ß and IL-17α are characteristic feature of rapid carcinogenesis in these models (111, 139, 177). Moreover, anti-cancer effects of FFA2 are not limited to colon. BaF3 leukemic cell proliferation in liver of mice could be countered by activation of FFA2 expressed profusely on BaF3 cells (22, 116). However, these studies are contended by a report demonstrating oncogenic properties of FFA2 (67). Using *in vitro* focus formation assay and *in vivo* tumorigenicity assay in nude mice, this report showed that expression of FFA2 transcript and protein is increased in digestive tract tumors (67). Thus, further investigations are warranted to understand whether FFA2 is a tumor suppressor or oncogenic in nature. Unlike FFA2, a role of FFA3 in cancer has not been reported probably because of its low expression compared to FFA2 in cancer cell lines (227) or alternatively due to its pro-cancer effects *in vitro* (219).

Role of FFA2 and FFA3 in metabolic regulation via actions in the gut—Both these receptors (FFA2 and FFA3) also affect metabolic functions related to their expression in the intestine. Aspects of this role for the receptors, like their role in immune modulation, are also debated. An interaction of SCFAs and these receptors on enteroendocrine cells or enteric neurons results in metabolic regulation through neural and/or gut derived hormonal mechanisms. Initially, FFA2 and FFA3 both were reported to mediate GLP-1 release from L cells with a greater role surmised for FFA2 (201). Low basal serum and colonic levels of active GLP-1 protein corresponded with FFA2 deficiency in mice, and this was suggested to be reflected in glucose intolerance in an oral glucose tolerance test and reduced GLP-1 secretion upon intra-colonic SCFA infusion or with primary colonic cultures from FFA2 KO mice (154, 201). Through this role of secreting GLP-1, FFA2, and possibly FFA3 are suggested to contribute to glucose homeostasis by improving/preserving insulin secretion and limiting insulin resistance (209). However, the role of FFA2, in particular, is debated by some studies with no difference in GLP-1 levels, basal or glucose stimulated observed between FFA2 KO and WT mice (56, 151, 192).

Another means by which these receptors may affect overall energy homeostasis is through modulation of PYY secretion. PYY lowers the intestinal transit rate and enhances satiety (17). By means of receptor KO mouse models and *ex vivo* colonic cultures, both FFA2 and FFA3 were documented to enhance PYY secretion and reduce gut motility (31, 52, 154, 165, 196). Another variable, for FFA3, is that it is also expressed in mouse and human sympathetic nervous system (93, 132) and it can, in part, mediate gut-brain axis communication (63). For example, a study showed that through gut-brain circuitry, FFA3 could enhance intestinal gluconeogenesis in mice by increasing expression of gluconeogenic enzymes. This effect was blocked by FFA3 antagonism (43). In sum, cumulative role of FFA2 and FFA3 on intestinal epithelial barrier integrity, immune cells, and gut hormone secretion suggests an important novel link between gut inflammation, diabetes, obesity, and SCFAs through these receptors. And how this link holds potential for new therapies against obesity and diabetes still remains to be investigated.

Role of FFA2 and FFA3 in extraintestinal tissues

Insulin secretion is a response to nutrient absorption. Nutrients, primarily glucose and free fatty acids, are well recognized to enhance insulin secretion (149). SCFAs have been suggested for multiple decades to modulate insulin release from β cells (141, 173, 200, 222). More recently, various groups have assessed the role of FFA2 and FFA3 in SCFAs mediation of insulin release (56, 120, 150, 151, 192, 207). Through these studies, FFA3 has emerged as a negative modulator of insulin secretion owing to its $G\alpha_{i/o}$ coupling (Fig. 4) (150, 192, 207). Consequently, FFA3 KO mice (192) *[Priydarshini et al., unpublished data]* and FFA3 overexpressing transgenic mice (207) exhibit complementary changes in glucose tolerance upon high-fat diet feeding without effects on insulin sensitivity suggesting that this phenotype is primarily a β cell secretory phenotype. Studies exploring the role of FFA2 on GSIS, however, have resulted in divergent outcomes [reviewed in (152)]. Two studies concluded that FFA2 activation enhances insulin secretion *ex vivo* as well as *in vivo* (120, 151) through $G\alpha_{q/11}$ pathway. Contrary to this, another study showed that acetate, which these authors suggested is produced in the islets, acts in an autocrine manner inhibiting GSIS through activation and $G\alpha_{i/o}$ coupling of FFA2 and FFA3 (192). While in human islets, acetate and synthetic ligands of FFA2 evoked $G\alpha_{i/o}$ or $G\alpha_{q/11}$ pathway either by increasing (151), decreasing (192) or no net effect on GSIS (120, 151). Groups supporting FFA2-$G\alpha_{q/11}$ coupling in islets showed further that FFA2 signaling maintains its insulinotropic effect and promotes compensatory β cell mass expansion under diet-induced obesity and pregnancy (56, 120, 151). In fact, FFA2 signaling is necessary for establishment of β cell mass. Results from our laboratory show that FFA2 KO mice have reduced β cell mass at birth and this is maintained during adulthood (both in male and female mice) (56, 209). As suggested by our experimental data, akin to its role in regulation of immune cell number, β cell FFA2 regulates aspects of β cell function and survival (209). However, prior to any clinical translational studies, more work is required to clarify these different outcomes between studies.

Besides insulin secretion, FFA2 has been reported to also affect insulin action (94). Using tissue specific overexpression model for this receptor, Kimura et al., showed that FFA2 in the white adipose tissue inhibits insulin mediated fat accumulation, promotes energy

homeostasis by enhancing energy expenditure and systemic insulin sensitivity in mice on high fat diet (94). These effects of adipose tissue-FFA2 overexpression were taken to suggest that overstimulation of the receptor is preventive against spontaneous or diet-induced obesity observed in FFA2 KO mice (94). While results from our laboratory and others have shown that FFA2 KO does not lead to higher body weight gain on high-fat diet (23,120,151), each of these studies show divergent outcomes for FFA2-KO effects on systemic insulin sensitivity, reporting either increase (23), decrease (94), or no change (120, 151). These former studies (23, 120, 151) support a role for FFA2 in white (44,68) as well as brown adipogenesis (70). Differing effects of FFA3 on body fat mass are also reported with FFA3 KO exhibiting reduced (165), enhanced (18) or similar (201) body fat mass. Thus, effects of these receptors on adiposity are far from clear but there is strong likelihood of these receptors playing a significant role through modulation of hormones affecting energy balance [leptin and ghrelin secretion by FFA2 (49,228)], satiety [PYY secretion by FFA3 (165), FFA2 [(31, 52, 154)], yet unidentified effects on CCK [(131) (39)] and sympathetic outflow [FFA3 (78, 93)]. Taken together, these receptors modulate secretion of insulin from the β cells directly or indirectly through GLP-1 secretion from L cells (described in the previous section) and mediate its effects on insulin-sensitive tissues like adipose (Fig. 4) (152). How all these peripheral actions of SCFAs work together is beginning to be understood, and it is increasingly apparent that FFA2 and FFA3 are participating in interorgan cross talk mediating the actions of SCFAs (33).

Implications for therapeutic use of FFA2 (and FFA3)

Based on findings reported earlier, gut FFA2 may be a befitting target for promotion of intestinal health and immune homeostasis. Several possibilities exist here and could be leveraged. First, acetate has highest concentration in the gut as well as in systemic circulation (Fig. 3) (96) suggesting that immune homeostasis possibly in the gut could be regulated naturally through consumption of fiber rich diet (111, 115, 181). Next, because functional redundancy of SCFA receptors does not allow partitioning of their physiological effects when using natural ligands, synthetic agonists could be of potential use to circumvent this issue. Third, FFA2 can exert effects both at intestinal epithelial and immune cells, where specific targeting of epithelial cells over immune cells or vice versa through synthetic agonists can be useful in different physiological settings.

Despite the compelling observations from *in vivo* (rodent) and *in vitro* (human and rodent cell lines) models, the first phase 2 clinical trial conducted in 2013 using FFA2 synthetic antagonist -[[(R)-1-(benzo[b]thiophene-3-carbonyl)-2-methyl-azetidine-2-carbonyl]-(3-chloro-benzyl)-amino]-butyric acid (GLPG0974, reported by Galapagos NV, Mechelen, Belgium; (28, 127)) failed to provide benefit to ulcerative colitis (UC) patients. This compound had been previously shown *in vitro* in human cell models to reduce FFA2 induced Ca^{2+} mobilization and neutrophil migration (28). Premise for using FFA2 antagonist in this 4-week study was that excessive neutrophil migration to the gut drives IBD and FFA2 is the neutrophil-SCFA receptor promoting neutrophil infiltration of the affected tissue (Figs. 4 and 5) (146,191). While GLPG0974 did reduce neutrophil activation and migration in UC patients, this decline did not produce any clinical benefits.

Failure of this trial signifies that our understanding of the complex interaction between FFA2 signaling and immune system may still be insufficient. First, most of our knowledge on the role of FFA2 in intestinal immunity is from mouse studies (92, 111, 116, 117, 181) and there may be differences in the type of inflammatory response elicited at FFA2 between species (7). Further, it has been shown that human FFA2 has constitutive activity (76), and how this constitutive activity is important in normal state and is altered in diseased states is yet unexplored. Also, for this trial, *in vivo* concentration of SCFAs, bacterial populations, and diet of the study population could also have influenced the results. Moreover, whether neutrophil activation improves or deteriorates the course of IBD is unclear (53). Additionally, FFA2 is expressed on different immune cell types; hence, consequence of its activity on different immune cell populations may vary along with differing effects based on existing (patho)physiological conditions (126). Lastly, as noted before, different ligands behave differently at FFA2 between the species [e.g., GLPG0974 is inactive at the mouse ortholog of the receptor (172) and CPTB stimulates $G\alpha_{i/o}$ at mouse FFA2 but $G\alpha_{q/11}$ at human FFA2 (151)] and therefore, our understanding of this FFA2 antagonist pharmacology may be incomplete [reviewed in (184)].

As apparent, the role of these receptors needs to be definitively described, to resolve the doubts associated with their function (Fig. 6). Novel synthetic ligands active at these receptors are being generated [reviewed in (122)]. However, caution should be exercised with their use and interpretation in biological models until an understanding of downstream effectors is known. Also, understanding if these are allosteric or orthosteric agonists needs to be detailed in interpreting studies (26, 62, 72, 120, 122, 151). Thus, the effect of novel ligands as they are generated should be assessed in different biological models *in vitro, ex vivo,* and *in vivo.* Results should be deduced relative to a standard control model. For example, in *in vitro* systems and primary cells, the stage of cell differentiation and time of harvest of tissue from the animal can cause variation in receptor expression and activity (44, 94, 120). To draw a corollary for human receptors, parallel experiments should be conducted in human cell lines, miniature organs derived from human biopsy tissue (4), or related tissues (151). Awareness of the *in vivo* models specifically the genetic backgrounds, diet composition, and gut microbiota of mice also needs to be considered (123, 191). To reduce variable outcomes, comparisons should also be made to gnotobiotic, same and nonidentical strains of mice fed diets with similar compositions (as we enumerate in detail in the section on “Interplay of Gut Microbiota, Diet, and Short Chain Fatty Acid Receptor Signaling”).

Most of the studies described in sections above have utilized global KO models. Considering their overlapping expression and ligand profiles, its imperative to develop double receptor KOs, and tissue specific receptor KOs. For example, for elucidation of their role specifically in intestinal epithelial and immune cells, gut epithelial specific Cre and myeloid specific Cre expressing mice may prove useful to generate mutant mice lacking these receptors specifically in IECs and leukocytes. Problems associated with analysis of human receptor pharmacology and function in physiological settings (which may differ greatly in comparison to the mouse ortholog) requires “humanized mice” expressing human receptor globally or under specific tissue promoter to be developed. The human receptor in such models can also be tagged with reporter protein (e.g., mCherry) or have its active site mutated (73, 131, 206). These tagged receptor models could be useful to explain receptor

dynamics and track expression with respect to physiological conditions. On the other hand, active site mutated human receptor is a thoughtful way to distinguish the effects of endogenous as well as synthetic ligand activity at the native and mutated receptor, respectively (73, 206). Such humanized mice when germ free, colonized with specific bacterial strains and normal or diseased human gut microbiota may also prove useful to study association of normal/altered gut microbiota with receptor activity and expression. In addition, studies with human enteroids could also increase our understanding of the role of these receptors. As apparent, unraveling the therapeutic potential of FFA2 (and FFA3) entails further in depth research.

GPR109a

Discovery—In one of the first studies, Benyo et al. (19) reported the expression of GPR109a in macrophages, other immune cells, and in adipocytes. Interestingly, these early studies uncovered that this GPCR was regulated by niacin, a well-known lipid-lowering agent (19). Moreover, the well-described side effect of flushing (which has historically limited its clinical usefulness) was indicated to be mediated through this GPCR. Another receptor GPR109b though closely related by sequence homology to GPR109a was also described; however, it showed much lower affinity to niacin (230). Moreover, this receptor, GPR109b, has thus far been described to be activated by aromatic D-amino acids and also molecules more similar to medium length fatty acids (135).

GPR109a is unique from FFA2 and FFA3, in that it is activated by the longer SCFAs, in particular butyrate, pen-tanoate and hexanoate, but it is also shown to be activated by beta-hydroxybutyrate (BHB) (135, 189). Due to the discovery of this GPCR as a niacin receptor and its well-defined side effects, multiple agonists have been developed with the goal of limiting this challenging side effect of flushing. These agonists largely appear to address the limitation of clinical use of niacin. For example, Richman et al. (159) described a number of pyrazole GPR109a agonists that seem to limit the flushing response to nicotinic acid, by preventing internalization of the receptor or activation of ERK 1/2 mitogen-activated protein kinase phosphorylation.

Expression—In addition to immune cells and adipose tissue (Fig. 3), GPR109a has also been shown to be expressed in pancreatic β cells (215). Though expressed poorly in pancreatic β cell culture lines, it is expressed in both mouse and human β cells within islets. GPR109a is also expressed in human microvas-cular endothelial cells (77) and microglial cells (54, 55). In retinal epithelial cells, it may have an anti-inflammatory role in diabetic retinopathy (58). GPR109a is also expressed in the lumen-facing apical membrane of colonic and intestinal epithelial cells, and is activated by butyrate (198). Interestingly, GPR109a has also been implicated in blood pressure regulation due to its expression in the brain, specifically in the rostral ventrolateral medulla (158). Additionally, GPR109a is expressed in mammary tissue (48), and may play a role in breast tumorigenesis, as noted in the following text. Compared to mammary tissue, its expression in breast cancer samples and cell lines is silenced, and ectopic expression of the receptor in these cell lines leads to cellular apoptosis. Consistent with this, in mice with GPR109a deletion, increased tumori-

genesis has been observed (48). Taken together, GPR109a is expressed in several organs, and appears to contribute to a host of metabolic responses.

Pharmacology/signaling—Signaling by GPR109a has been predominantly shown to occur via $G\alpha_{i/o}$ pathway (215) (Figs. 1 and 4), which leads to lower intracellular cAMP levels. In adipose tissue, this results in reduced lipase activity, plasma triglycerides, and free fatty acid levels. In islets, GPR109a activation reduces insulin secretion and interestingly, its expression is significantly decreased in islets from diabetic patients and murine diabetic models (db/db mice) (215). However, the significant role of GPR109a in the prevention of diabetes needs further investigation. Cell surface levels of GPR109a have been shown to be tightly regulated. In depth analysis of GPR109a internalization process (104) has revealed that it is readily down regulated through clathrin-coated pathways (Fig. 2D). Further, its agonist-dependent internalization is regulated by arrestin-3 and G-protein coupled receptor kinase-2 (GRK2). In an intriguing study, Mandrika et al. (114) showed that GPR109a forms homodimers with itself and also heterodimers with GPR109b, and the dimerization process is not influenced by ligand binding. In this study, the authors described that GPR109a dimerization process occurred prior to it reaching the plasma membrane, possibly during receptor synthesis and might have a role in receptor trafficking (Fig. 2I). As both GPR109a and GPR109b are expressed in adipose tissue and regulate lipolysis, it remains unclear whether homo and/or heterodimer formation of these receptor subtypes plays an important role in their function in adipose tissue.

Role of GPR109a in the gut physiology

Role in nutrient sensing—The nutrient sensing machinery of the intestine includes the trans-membrane solute carrier transporters (SLC) in addition to the GPCRs being described here. While we do not know much about the relationship between SLC and GPCRs, one study examined this interrelationship. In colonic epithelial cells, SCFA uptake is well described to be mediated by luminal membrane MCT-1 (monocarboxylate transporter-1), where butyrate is known to stimulate MCT-1 expression. Borthakur et al. (30) recently demonstrated that GPR109a functions as a novel nutrient sensor, where short-term butyrate treatment (30–120 min) enhanced MCT-1 function and surface membrane expression through GPR109a signaling. Importantly, this increase in MCT-1 function and expression occurred at least in part through cAMP inhibition via GPR109a signaling by $G\alpha_{i/o}$ pathway (30).

Role of GPR109a in intestinal inflammation—Butyrate-activated GPR109a signaling has been reported to have an essential role in anti-inflammatory effects in the intestine. More specifically, GPR109a KO mice were shown to be more susceptible to DSS-induced colitis by showing increased mortality compared to control (181). Moreover, GPR109a KO mice also had increased susceptibility to colitis when subjected to Azoxymethane (AOM) plus DSS-induced colonic inflammation and carcinogenesis (176). Pro-inflammatory markers such as IL-6, CXCL1, CCL2, IL-17, and IL-1ß were each elevated and anti-inflammatory IL-18 was decreased in colons of GPR109a KO mice compared to WT mice (176). Using GPR109a KO mice and bone marrow chimera models, Macia et al. demonstrated the importance of these receptors in gut homeostasis. Additionally, it was observed that

GPR109a-activated NLRP3 inflammasome, which promoted gut epithelial integrity (111). The role of GPR109a in the suppression of colitis thus appears partially to be due to the activation of NLRP3 inflammasome, decreased pro-inflammatory markers and increased IL-18 production in colonic tissue (Fig. 5).

Anti-inflammatory action of GPR109a in colon mainly involves increased production of anti-inflammatory Treg and IL-10 producing $CD4^+$T-cells. $CD4^+$ Treg cells suppress the deleterious activities of T-helper cells and thus play a critical role in immune mechanisms (163). Butyrate is considered to have a strong impact on the induction of Tregs as mice fed with butyrate displayed increased number of Treg cells in the colon compared to other SCFAs such as acetate and propionate. Germ free mice have lower Tregs than conventionally raised mice and high-fiber diet induces greater number of Treg cells than low-fiber diet (57). GPR109a is activated by butyrate and niacin and this signaling has been observed to regulate the production of Treg cells, and IL-18, and IL-10 producing T cells in colon. Colonic lamina propria of GPR109a KO mice also showed reduced frequency of Treg cells, IL-18, and elevated levels of IL-10 and IL-6 compared to WT littermates. However, no difference was observed in the spleens of WT and GPR109a KO mice, signifying that the action of GPR109a may be specific to the colon (111,176). The authors of this study further provided evidence that GPR109a-dependent differentiation of Tregs played an essential role in colonic inflammation as colonic dendritic cells and macrophages from GPR109a KO mice were defective in inducing differentiation of native T cells into Tregs compared to their WT counterparts. In other related studies, Chang et al. reported some of these aforementioned properties to be related to the inhibition of HDACs as a potential mechanism (36). However, whether SCFA-mediated GPCR activation promotes Treg frequency in intestinal lamina propria or impacts T-cell differentiation in lymph nodes remains unclear and further studies with GPR109a KO mice are needed to clarify this issue. Overall, data from multiple independent laboratories indicated that GPR109a-mediated induction of Tregs in colonic dendritic cells and macrophages to be a potential mechanism by which GPR109a regulates anti-inflammatory actions in colon.

While it is clear GPR109a senses SCFAs, it is intriguing that BHB also activates this receptor. As well known, BHB increases during fasting, and other related conditions, such as low calorie or ketogenic diet consumption, where these conditions are known to have metabolic benefits, including diminished inflammation. In fact, it has been suggested that BHB under conditions of fasting inhibits lipolysis through GPR109a and BHB-induced GPR109a activation on monocytes and macrophages reduces neuroinflammation (55,135). Whether the anti-inflammatory effects of BHB-GPR109a interaction are relevant in intestinal inflammation or underlie ketogenic diet induced anti-inflammation needs verification.

Role of GPR109a in colon cancer—In one of the first reports describing this receptor in 2009 (198), GPR109a was observed to be expressed on the luminal plasma membrane of the epithelial cells in colon and small intestine. Interestingly, in this report, GPR109a was found to be downregulated in human colon cancer cell lines (along with mouse models of colon cancer). Moreover, expression of GPR109a in these cells led to apoptosis in the presence of its ligands. These expression patterns were further confirmed in mice, and were

observed to be downregulated in germ free mice (38). Using two different models of carcinogenesis (AOM plus DSS-induced carcinogenesis and $Apc^{Min/+}$-driven intestinal and colon carcinogenesis), Singh et al. demonstrated that GPR109a KO mice were more prone to development of colon cancer than WT mice as GPRKO;$Apc^{Mm/+}$ mice had strikingly more polyps in the colon and small intestine than did $Apc^{Mm/+}$ mice (176). GPR109a levels have also been observed to be downregulated in human and mouse models of colon cancer and in cancer cell lines possibly due to DNA methylation (198). Restoration of GPR109a in cancer cell lines induced apoptosis only in the presence of ligands, for example, niacin and butyrate (198). Apoptosis induced by overexpression of GPR109a in cancer cell lines was not due to inhibition of HDACs but involved inhibition of Bcl-2, Bcl-xL, and cyclin D1, NFκb activity, and upregulation of death receptor pathway (198). Moreover, Bardhan et al. showed that IFN-γ-activated transcription of GPR109a promoter in colon carcinoma cells independent of DNA methylation, leads to its increased transcription, concluding that GPR109a acted as a tumor suppressor in colon cancer by IFN-γ regulation of transcription (15) (Fig. 2H). In summary, it appears that GPR109a plays important roles in gut inflammation and nutrient sensing in the gut, and may have a role in carcinogenesis.

Role of GPR109a in extraintestinal tissues

Role in diabetes—Suggesting a role in diabetes, GPR109a has been observed to be down regulated in islets of diabetic db/db mice as well as in type 2 diabetes (T2D) patients compared to islets of normal control subjects (215). Additionally, in Min6 cells (a mouse pancreatic β cell line), high glucose, plus nicotinic acid or 3 hydroxy butyrate treatment inhibited GPR109a expression (225). Administration of selective GPR109a agonist, GSK256073, in diabetic human subjects significantly reduced serum glucose and nonesterified fatty acids (NEFA) without any complications of flushing and GI disturbances (45). Taken together these studies indicate a potential role of GPR109a in T2D and promising therapeutic value of GPR109a agonist, GSK256073 in the treatment of T2D.

Role in atherosclerosis—Niacin reduces the progression of atherosclerosis by lipid modifying effects. Niacin treatment also significantly reduced the expression of inflammatory markers such as TNFα, IL-6, IL1β, and IL12p40 in LPS-stimulated macrophages from WT mice but not GPR109a KO mice (229). Also, the expression of cholesterol transporter ABCG1 and cholesterol efflux was reduced by niacin in macrophages from GPR109a KO mice compared to WT mice. In addition, activation of GPR109a by niacin reduced macrophage recruitment to atherosclerotic plaques. These reports provide compelling evidence that nicotinic acid reduces the progression of atherosclerosis via activation of GPR109a on immune cells and regulation of inflammation (109).

Therapeutic potential of GPR109a agonists

As described earlier, the antiatherogenic and antiinflammatory effects of GPR109a are not only via antilipolytic effects but also through the role of GPR109a in immune cells. The reduction in the progression of atherosclerosis was attributed to the reduced infiltration of vessel wall by GPR109a expressing macrophages and neutrophils. There is also evidence that dimethyl fumarate (DMF) and dietary metabolites such as butyrate mediate antineuroinflammatory effects in Parkinson's disease and multiple sclerosis, respectively, via

activation of GPR109a (83). DMF has also been shown to have a beneficial effect in the treatment of psoriasis (12, 125). In intestine, butyrate-mediated activation of GPR109a leads to improvement in colitis and limits progression of colon cancer. In summary, many studies have implicated GPR109a agonists as potential therapeutic targets in the treatment of atherosclerosis, neuroinflammatory disorders, colitis, and psoriasis; however, more detailed mechanistic studies are warranted to further establish the direct role of GPR109a in these processes.

Olfr78

Discovery—Olfactory Receptor 78 (Olfr78) also known as OR51E2 in humans (Olfactory receptor family 51, subfamily E, member 2) or PSGR, (prostate-specific G-protein-coupled receptor) is expressed, in multiple tissues (148, 160, 221) (Fig. 3). As compared to FFA2, FFA3, and GPR109a, Olfr78 is overall less well characterized regarding its expression, function, and pharmacology. Although primarily expressed in olfactory tissues, recent reports indicate its expression in other organs. Initial reports observed Olfr78 as a gene upregulated in prostate cancer (224), as such, it was initially called prostate-specific G-protein-coupled receptor. While it was initially observed to be expressed in human prostate tissue, expression in the human brain was also found, specifically in the medulla oblongata and olfactory region. In mice, it was also observed to be expressed in the colon and in rats, expression was observed in the liver (51, 118).

Pharmacology/signaling—Acetate and propionate have both been shown to activate Olfr78, with EC_{50} values for acetate around 2.4 mmol/L and for propionate ~0.9 mmol/L (Table 3) (148). Regarding its signaling pathway, the early reports suggest it is likely that Olfr78 is a $G\alpha_s$-coupled receptor, as cAMP levels increase when it is activated (148). In melanocytes and prostate cells, coupling through $G\alpha_q$ has also been reported. However, to the best of our knowledge, the G-protein signaling preferences of Olfr78 have not been extensively explored. Moreover, other characteristics of its signaling mechanisms have not been examined to date.

Role of Olfr78 in the gut physiology/ pathophysiology

As compared to other SCFA receptors, the role of Olfr78 in GI physiology is largely unknown. Thus far, it has been reported that elevated levels of OR51E2/Olfr78 are observed in colon cancers (2, 101, 107). It is also identified as one of the candidate driver genes for microsatellite-unstable colorectal cancer with a somatic mutation frequency of >20% (2). Olfr78 is expressed in colonic epithelial cells, and specifically a class of enteroendocrine cells that coexpress PYY (51). However, its role in these cells is not known and needs further investigation.

Role of Olfr78 in extraintestinal tissues

Recent evidence suggests that Olfr78/OR51E2 plays an important role in nonolfactory organs. For example, this receptor is implicated in blood pressure regulation (147) and a variety of other cellular functions. Pluznick et al. showed that Olfr78 activation increased whereas FFA3 activation was able to reduce blood pressure (148). In another study, Chang et al. reported an activation of Olfr78 by lactate in carotid body under hypoxic conditions,

which resulted in elevated blood pressure thereby preventing hypotension and restoring normal blood pressure (35). In prostate cells, overexpression of OR51E2 induced chronic inflammatory response leading to prostate intraepithelial neoplasia (160,161). Ligand-induced activation of OR51E2 has also been shown to affect melanocyte proliferation, differentiation, and melanogenesis (60). Overall, these studies indicate a pathophysiological role of this receptor in blood pressure regulation, colon cancer, and prostate neoplasia. As there are only limited studies thus far, further studies are needed for a thorough analysis of its functional significance in different organs.

Interplay of Gut microbiota, Diet, and Short Chain Fatty Acid Receptor Signaling

So far, the interaction between the receptors and the SCFAs has been described. However, gut microbiota, the major site of SCFA production, can also exert control on the receptors by modulating the concentration of their ligands (69, 142). But this relationship between the gut microbiota and receptors through SCFAs is far from simple. Gut microbial structural components and other metabolites also affect host physiology with potential bearings on SCFA levels and these receptors [reviewed in (169, 199)]. Gut microbiota can also interact with the host genotype, and it can be influenced by hygiene habits (191, 199, 218). Furthermore, it is well documented that mammalian diet influences the compositional properties of the gut bacteria (182). Both these processes along with dietary fiber content govern SCFA production in the gut (96, 110). Of note, intestinal inflammatory diseases like IBD involve complex interaction of all of the above (16, 153). With this obligatory relationship between diet, gut bacteria and SCFAs, involvement of SCFA receptors in dietary intervention induced protection against gut barrier breach and inflammation is possible, and is slowly being experimentally tested.

In WT or gnotobiotic mice, SCFA supplemented drinking water and/or fiber/SCFA enriched diets have been employed to understand the interplay of gut microbiota, diet, and short chain fatty acid receptor signaling in promotion of intestinal health and its discontinuity in intestinal inflammation. From these studies, FFA2 and GPR109a have emerged as links between host immune responses and gut microbiota. These receptors are considered to act as sensors of these gut microbial metabolites, the SCFAs, and maintain intestinal immune homeostasis. A loss of the signals or of the receptors then would be causal for intrinsic immune defects and deregulated immune response to exogenous insults. In fact, SCFA deficit arising from the absence of gut microbiota (as in germ free mice, antibiotic-treated specific pathogen free mice, and antibiotic-treated WT mice) or lack of fiber in the diet reduces colonic Treg cell population and exacerbates DSS colitis in mice (1, 11, 111, 116,157, 176, 181). SCFAs, niacin, or pharmacologic agonist treatment could rescue this phenotype but only in the germ free and gut microbiota-modified mice (1, 116, 176, 181). The FFA2 KO and GPR109a KO mice lacked such protection (116, 176). As the level of SCFAs in gastrointestinal tract is to a large extent determined by fiber content in the diet, a high proportion of fiber should produce similar effects (69, 142). Consumption of fiber-free diet in WT mice worsened and of high fiber diet in FFA2 KO and GPR109a KO mice did not combat DSS colitis (111, 176). Dietary fiber also conferred protection against colitis-associated cancer in FFA2 and GPR109a-dependent manner (111, 176, 177). FFA2 and

GPR109a, thus, emerge as functional links between gut microbiota via SCFAs to intestinal immune effects (Fig. 7).

The mechanistic underpinnings of diet induced immune protection extend beyond SCFA-cognate receptor mediated upregulation of protective cytokines (IL-10, IL-18), inflammasome activation, and increase in colonic Tregs (effects discussed earlier) (111, 116, 176). Diet also can significantly modify gut microbial composition and function (41,119,182). Fiber-deficient diet has been observed to select for members of Bacteroidetes and Proteobacteria families which was associated with severe DSS colitis and reduced IL-18 levels (1, 111). Whereas, high-fiber diet, selected for specific protective members of Bacteroidetes *(Porphyromonadaceae* and *Rikenellaceae)* and Firmicutes *(Lachnospiraceae)* (111) and was found to be associated with reduced severity of DSS colitis and enhanced IL-18 levels. Colonization of germ free mice with high-fiber diet-induced gut microbiota could resist DSS colitis (111). An interesting observation in these studies was the expansion of colitogenic and cancer-associated bacteria (like Proteobacteria, *Prevotellaceae, Helicobacter hepaticus)* in FFA2 KO and GPR109a KO mice (111, 176–178). In the same line, presence of FFA2 was shown to be necessary for selection of *Bifidobacterium species* in high fiber diet fed mice (177). Of note, decrease in *Bifidobacterium species* and increase in *Proteobacteria* (represented mainly by *Escherichia coli*) was reported to be associated with intestinal inflammation and colon cancer (1, 10, 46, 134). Consistent with this, these receptor KO mice, which were susceptible to colitis, associated cancer (111,139,176,177), and could be rendered cancer resistant with use of antibiotics (177), suggests that gut microbiota alone could also confer beneficial effects. Indeed, transfer of “protective bacteria” to the deficient mice could confer protection against colitis regardless of receptor or dietary status (111, 177). Moreover, suppression of colon inflammation and carcinogenesis in FFA2 KO mice could be achieved by oral gavage of *Bifidobacterium infantis* (177, 178) or upon co-housing with WT littermates allowing transfer of protective bacteria from WT to KO mice (111). These results point to the fact that “beneficial bacteria” may confer protection against intestinal inflammatory diseases (137), while SCFAs and cognate receptors, thus, are not the entire story but only part of it. Their contributions are likely additive, to enhance the effects of beneficial microbes, and this needs further investigation.

The recent work of Macia et al. (111), Sivaprakasma et al. (177), and Singh et al. (176) alludes to the role of SCFA receptors in preventing disruption of commensal gut bacteria. Selection of specific gut bacterial profiles associated with absence of receptors (and their signaling) is intriguing. It suggests that receptor signaling is required for basal immune homeostasis and to balance the activities of harmful and beneficial bacteria that cohabit in the gut (170). This also suggests that receptor absence can influence tolerance with contribution from various factors like lack of IgA response (220). In one of our reports, we did not specifically observe any discrimination against cancer/colitis promoting bacteria in FFA2 KO as compared to WT mice (56) while McNelis et al. (120) observed selection of butyrate producers in WT and acetate producers in FFA2 KO mice which could be a compensatory response for lack of acetate receptor (FFA2) signaling. Though high-fat diet is associated with dysbiosis and mild inflammation (34), no inference was drawn regarding increase of intestinal inflammation promoting bacteria in the KO mice (120). While these

differences may arise from the distinct focus of these separate studies, future studies are required to understand the complex association of specific gut microbial species in these different KO models.

Gut microbiota dependence of SCFA receptor signaling has also been shown to influence metabolic roles of these receptors. For example, FFA3 plays a crucial role in gut microbiota-dependent host energy balance (165). Reduced adiposity in FFA3 KO mice compared to the WT mice was abrogated in germ free conditions. Using conventionally raised or germ-free mice co-colonized with two human gut microbes the Saccharolytic bacterium, *Bacteroides thetaiotaomicron,* and the methanogenic archaeon, *Methanobrevibacter smithii,* the authors demonstrated that co-colonization of germ free mice with these microbes significantly increased the levels of PYY but not in FFA3 KO mice (165). Additionally, intestinal transit rates were faster in FFA3 KO mice compared to WT littermates and this effect was abolished in germ free conditions. Thus, gut microbiota-dependent SCFA production and their signaling at FFA3 are necessary for PYY secretion (165). Similarly, spontaneous obese phenotype of FFA2 KO mice could be rescued under germ free conditions or upon antibiotic treatment (94).

Functional implications of these observations to humans are needed. For example, proof of concept experiments using “humanized mice” colonized with gut microbiota of healthy and IBD patients are needed. In such study, colonization of such humanized mice with healthy bacteria like Firmicutes [anti-inflammatory bacteria expanded on high fiber feeding (111)] was recently shown to be beneficial against colitis but the contribution of the SCFA receptors was not studied (128). To harness the potential of SCFA receptors as therapeutic targets, more detailed and focused studies are required.

Conclusion

SCFAs have been long known to be key nutrients generated in the gut, contributing to GI tract physiology and at times, pathophysiology. During the last 20 years, significant evidence has accumulated that cognate GPCRs, FFA2, FFA3, GPR109a, and Olfr78, serve as sensors for SCFAs. We are just beginning to understand the role of these receptors in health and disease, and this article outlines the observations so far in modulation of gastrointestinal health.

While, the role of FFA3 is still less appreciated and the studies on Olfr78 are in their infancy, we have a bit better understanding of the contributions of FFA2 and GPR109a. Leveraging genetic mouse models (receptor knockout and overexpression), synthetic ligands, latest technologies of gut microbiome profiling, recent studies have corroborated a role for FFA2 and GPR109a in diseases like IBD and colon cancer underscoring their role as potential therapeutic targets. However, there are a number of questions yet unanswered. With single receptor whole body knockouts, the function of these receptors can be compensated. In other words, in absence of the use of selective synthetic agonists *in vivo,* it is difficult to assign a specific role to just one of these receptors. SCFAs also have receptor-independent effects. Thus, future studies aiming to answer these questions will benefit from the use of more sophisticated mouse models like with double, multiple or tissue specific receptor knockouts,

and enteroids prepared from these mouse models and from human intestinal biopsies from healthy subjects and patients with IBD, colon cancer, or metabolic disorders.

In addition, FFA2 and GPR109a can signal through multiple pathways. Activation of one pathway may have beneficial or harmful effects that may also depend on time of activation during the course of disease (e.g., FFA2-dependent inflamma-some activation is beneficial in the gut but may be detrimental in gout (208)). Pharmacologic tools for targeted tissue specific activation of these receptors are thus required. These receptors lie at the crossroad of inflammation and metabolism. How these pathways intersect and influence the course of inflammatory and metabolic disorders through these receptors is intriguing. Furthermore, interdependence of gut microbiota, diet, and short chain fatty acid receptor signaling (with additional effects from host genetics, health status, and environment) may have distinct functional consequences on the host. Thus, several caveats exist to the pharmacologic and therapeutic translation of these receptors to humans. Nonetheless, this interplay of gut microbiota, SCFAs and cognate receptor signaling is the Pandora's promise of combating serious inflammatory diseases like IBD, colon cancer, and promoting gut health (Fig. 7).

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Didactic Synopsis

1. Short chain fatty acids (SCFAs) produced by gut microbial fermentation serve both as an energy source and signaling molecules.
2. Molecular sensors for these SCFAs are G-protein coupled receptors. These GPCRs, namely, FFA2 and −3 and GPR109a have immune as well as metabolic roles.
3. These receptors, including Olfr78, have overlapping ligand profiles and some functional redundancy, but their restricted expression pattern in various cell types in the gut (and other tissues) and distinct G-protein coupling profiles allows for specific physiologic effects.
4. FFA2 and GPR109a modify the gut immune cell repertoire and inflammatory responses regulating immune homeostasis, guard intestinal epithelial barrier against pathogenic intrusion and tumor growth.
5. FFA2 and FFA3 appear to regulate GLP-1, GIP, and PYY secretion exerting effects on glucose homeostasis.
6. These roles signify a link between gut microbiota and host physiology through SCFA receptors.
7. Targeted modulation of these SCFA receptors may promote intestinal health.

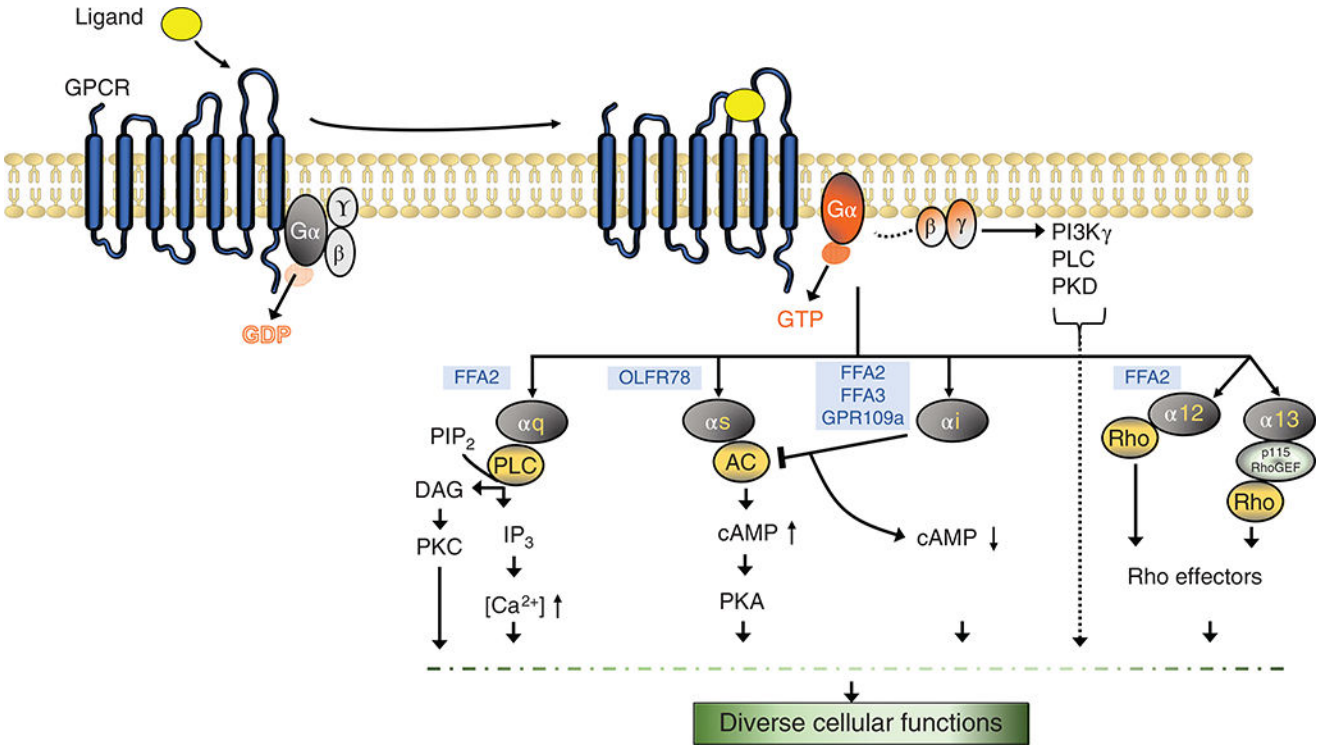

Figure 1.

Different G-protein signaling pathways. Ligand (agonist) occupation of the binding pocket in the GPCR leads to conformational changes in the receptor and an altered interaction with heterotrimeric G-proteins. The activated receptor acts as a guanine nucleotide exchange factor that catalyzes the exchange of GDP for GTP on the Gα subunit and induced dissociation of active Gα subunit and Gβγ dimer (activated subunits represented with orange shade). There are four main Gα subunit protein classes, Gαq, $G\alpha_s$, $G\alpha_i$, $G\alpha_{12/13}$. Each GPCR can couple with one or more Gα subunits. The activated Gα subunits can bind to and regulate the activity of several downstream effector molecules generating a cascade of signaling responses that culminate into specific cellular responses. Gαq/11 family members bind to and activate phospholipase C (PLC) which hydrolyses phosphotidylinositol 4,5-bisphosphate (PIP2) to diacylglycerol (DAG) and inositol triphosphate (IP3) which can mobilize calcium from intracellular stores or activate downstream protein kinases and modulate cell response. $G\alpha_s$ subunit activates adenylyl cyclase (AC) which increases intracellular cyclic adenosine monophosphate (cAMP) levels leading to activation of protein kinase A (PKA). Gαi subunit inhibits AC and reduces intracellular cAMP concentration. $G\alpha_{12/13}$ can activate Rho kinases. The Gβγ subunit can activate phosphoinositide 3 kinase γ (PI3Kγ), protein kinases (PKD), and phospholipases (PLC). Various short chain fatty acid receptors (highlighted in blue color) are listed based on their G-protein-coupling preference. FFA2/3, free fatty acid receptor 2/3; Olfr78, olfactory receptor 78; GPR109a, G-protein-coupled receptor 109a (32,135,145,147,152).

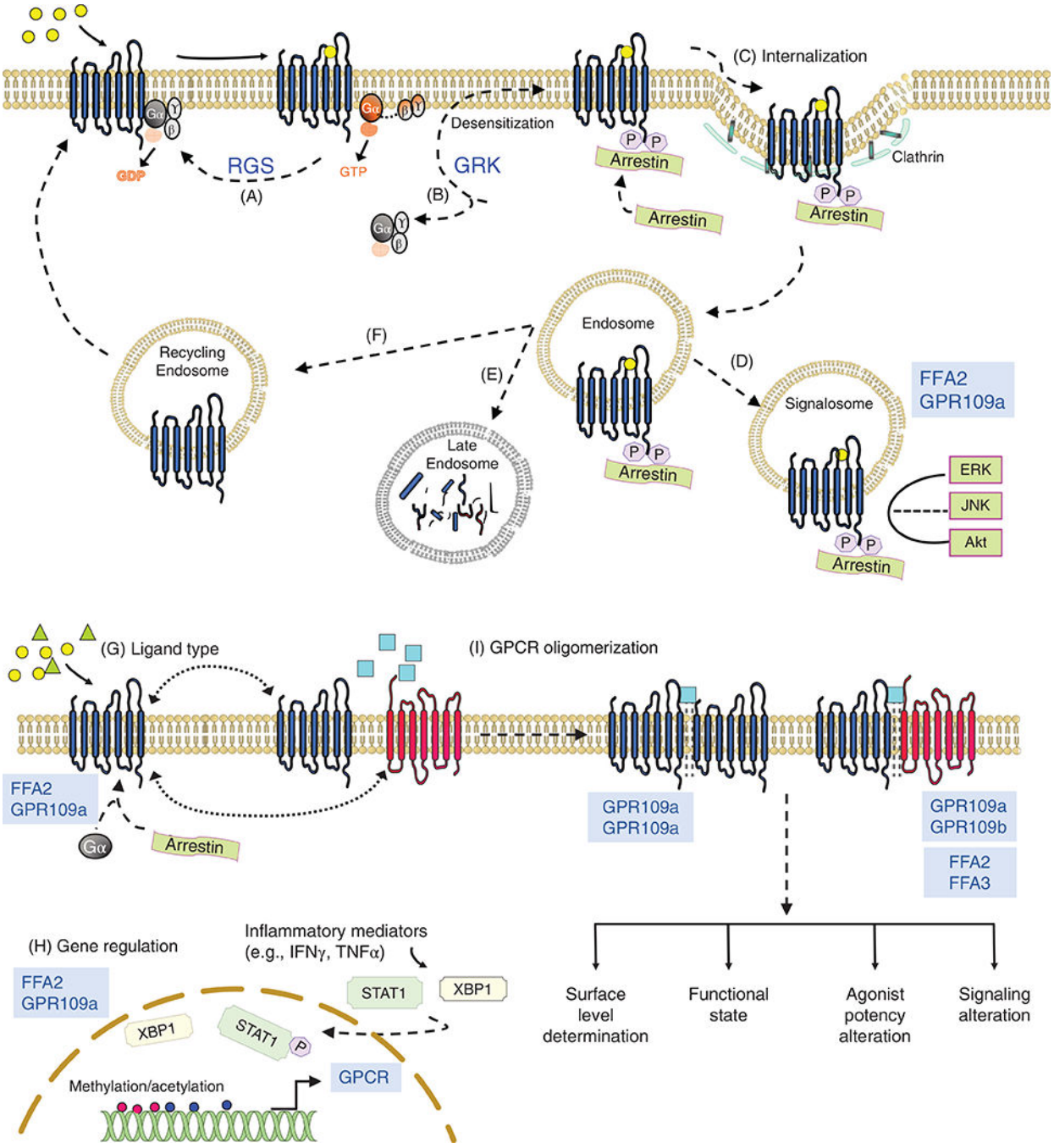

Figure 2.

Regulation of GPCR signaling. Binding of agonist to the GPCR initiates a series of events: exchange of GDP for GTP on Gα subunit, dissociation of activated GTP-Gα and Gβγ dimer, stimulation of downstream effectors (depicted in Fig. 1). To prevent overstimulation and assure receptor resensitization, GPCR signaling is regulated by multiple mechanisms (few are enumerated here; see Further Reading list for detailed description). (A) Regulator of G-protein signaling (RGS) proteins function as GTPase-activating proteins, enhance the intrinsic GTP hydrolysis activity of the Gα subunit resulting in generation of inactive GDP-

Gα, and promoting the reassembly G-protein heterotrimer and its reassociation with the receptor. (B) GPCR kinases (GRK) phosphorylate specific residues on the intracellular domain of agonist-occupied receptor. Phosphorylated receptor recruits β arrestin that sterically hinders interaction of the receptor with G-protein, thus uncoupling the G-protein complex from the GPCR (termed as desensitization). (C) Binding to the phosphorylated GPCR activates arrestin enabling it to perform ligand regulated scaffolding functions. Activated β arrestin interacts with the endocytic machinery (clathrin and adaptor proteins) and initiates receptor internalization. (D-F) Internalized GPCR in GPCR-β arrestin endosome can have multiple fates. GPCR-β arrestin complex can (D) direct the assembly of signalosome by allowing coupling of mitogen-activated protein kinases propagating further signaling pathways, (E) be targeted for lysosomal degradation, or (F) GPCRs can be trafficked to recycling endosomes and recycled back to the plasma membrane for resensitization. (G) Ligand type can also determine course of GPCR signaling where biased agonists can specifically activate either G-protein or β arrestin-mediated signaling pathway. (H) Gene transcription can also affect the total membrane levels of the GPCR. Transcription can be regulated by various inflammatory mediators, which can activate transcription factors that bind directly like XBP-1 on FFA2 promoter or indirectly affect chromatin accessibility by altering acetylation/methylation of the promoter (IFNγ for GPR109a). (I) GPCRs can form oligomers (homodimers or heterodimers) that can regulate (1) cell surface delivery following receptor maturation or cellular trafficking following agonist activation, and (2) receptor pharmacology and signaling. Short chain fatty acid receptors (highlighted in blue color font) are noted if data exist on their regulatory mechanisms. FFA2/3, free fatty acid receptor 2/3; GPR109a, G-protein-coupled receptor 109a (6, 8, 14, 15, 102, 112, 114, 120, 144, 179, 212).

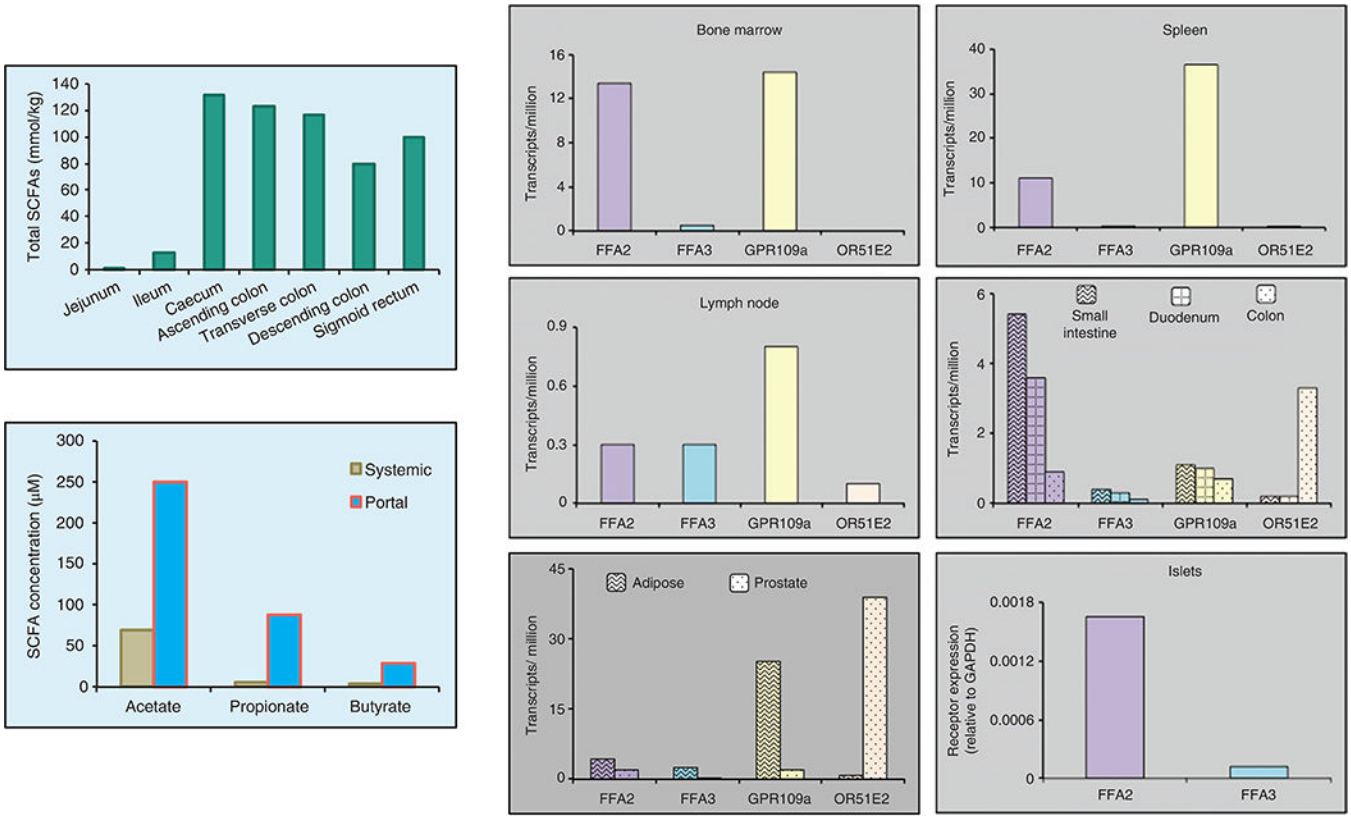

Figure 3.

Short chain fatty acid levels and expression of cognate receptors in select tissues. Short chain fatty acid concentrations in contents along the intestine (left upper panel) and systemic and portal blood in human subjects (left lower panel) [adapted, from Cummings et al. (40)]. Right-hand panel, human short chain fatty acid receptor expression in select tissues presented as plot of mean transcripts per million from HPA (human protein atlas) except for expression in human islets that was obtained by qRT-PCR and presented relative to GAPDH (Brian T Layden, unpublished data).

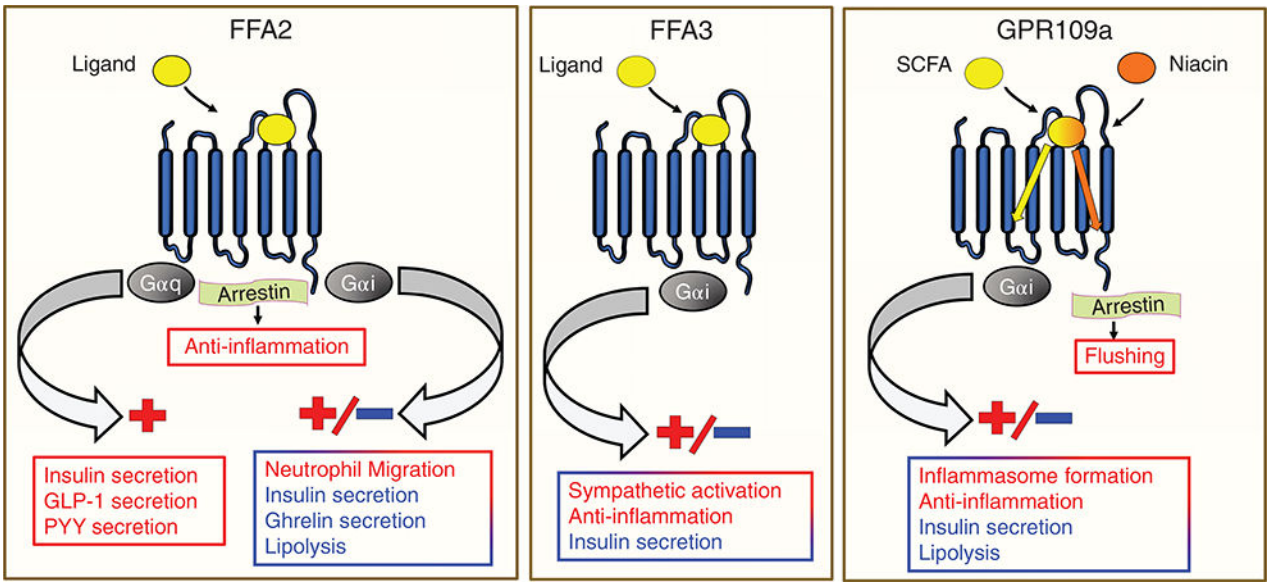

Figure 4.

Signaling pathways downstream of FFA2, FFA3, and GPR109a activation and corresponding effects. Apart from G-proteins, FFA2 can signal via arrestin exerting anti-inflammatory effects. Niacin at GPR109a shows biased agonism, arrestin signaling preferentially over G-protein signaling causing flushing.

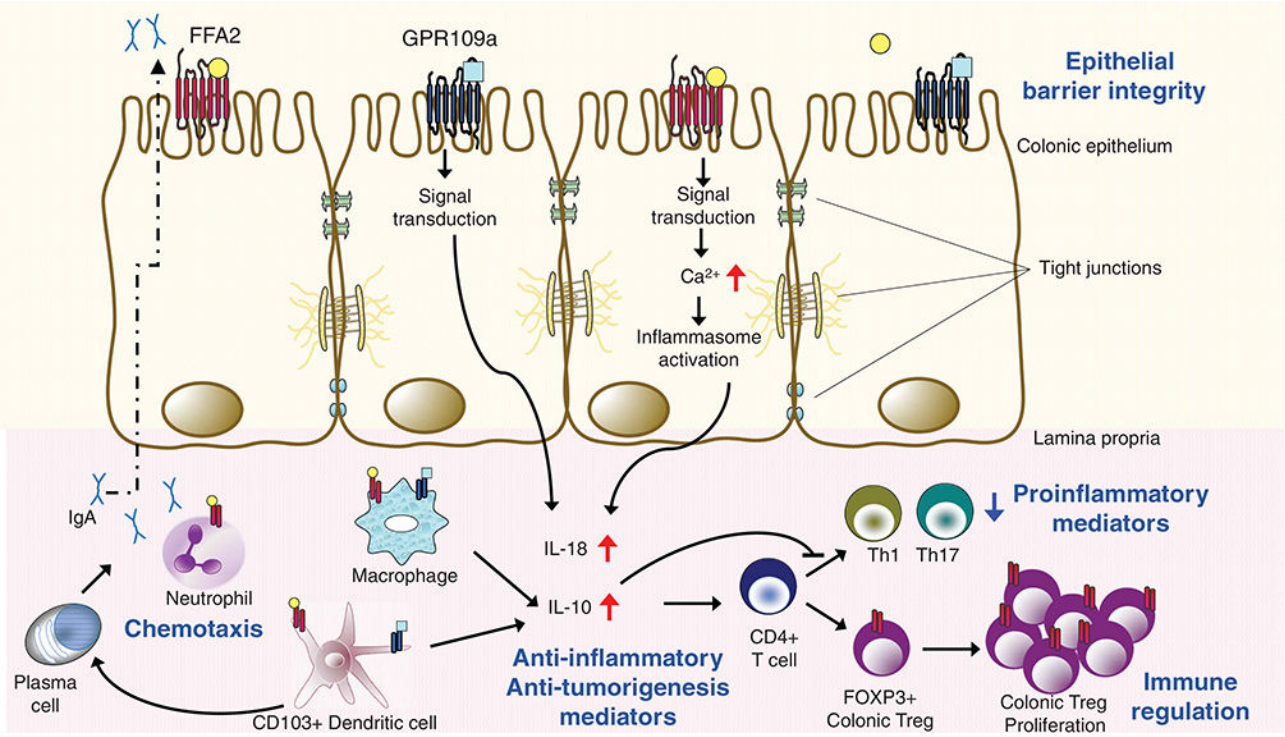

Figure 5.

Short chain fatty acid receptors in gut immune homeostasis. Short chain fatty acids produced by fermentative activity of gut microbiota bind and activate receptors on intestinal epithelial cells and immune cells like macrophages, neutrophils, and dendritic cells. Production of cytoprotective IL-18 (from FFA2/GPR109a-dependent activation of inflammasome), anti-inflammatory IgA (from FFA2-dependent activation of B cells), anti-inflammatory IL-10 (from FFA2 activation and GPR109a-dependent activation of macrophages and dendritic cells), and (FFA2-and GPR109a-dependent) differentiation and proliferation of Tregs protect against conditions leading to colitis and colitis associated cancer. Taken together, these processes influence epithelial barrier integrity, adequate neutrophil migration, balanced proinflammatory (Th1 and Th17), and immunosuppressive (Treg) responses under conditions of inflammatory insult. CD, cluster of differentiation; IgA, immunoglobulin A; Th, T helper cells; Treg, regulatory T cells; short chain fatty acids are represented byo, □; FFA2 and GPR109a, respectively, on immune cells are represented by I, I; red upward arrow signifies increase; blue downward arrow signifies decrease (92, 111, 116, 117, 176, 177, 181, 190, 191, 220).

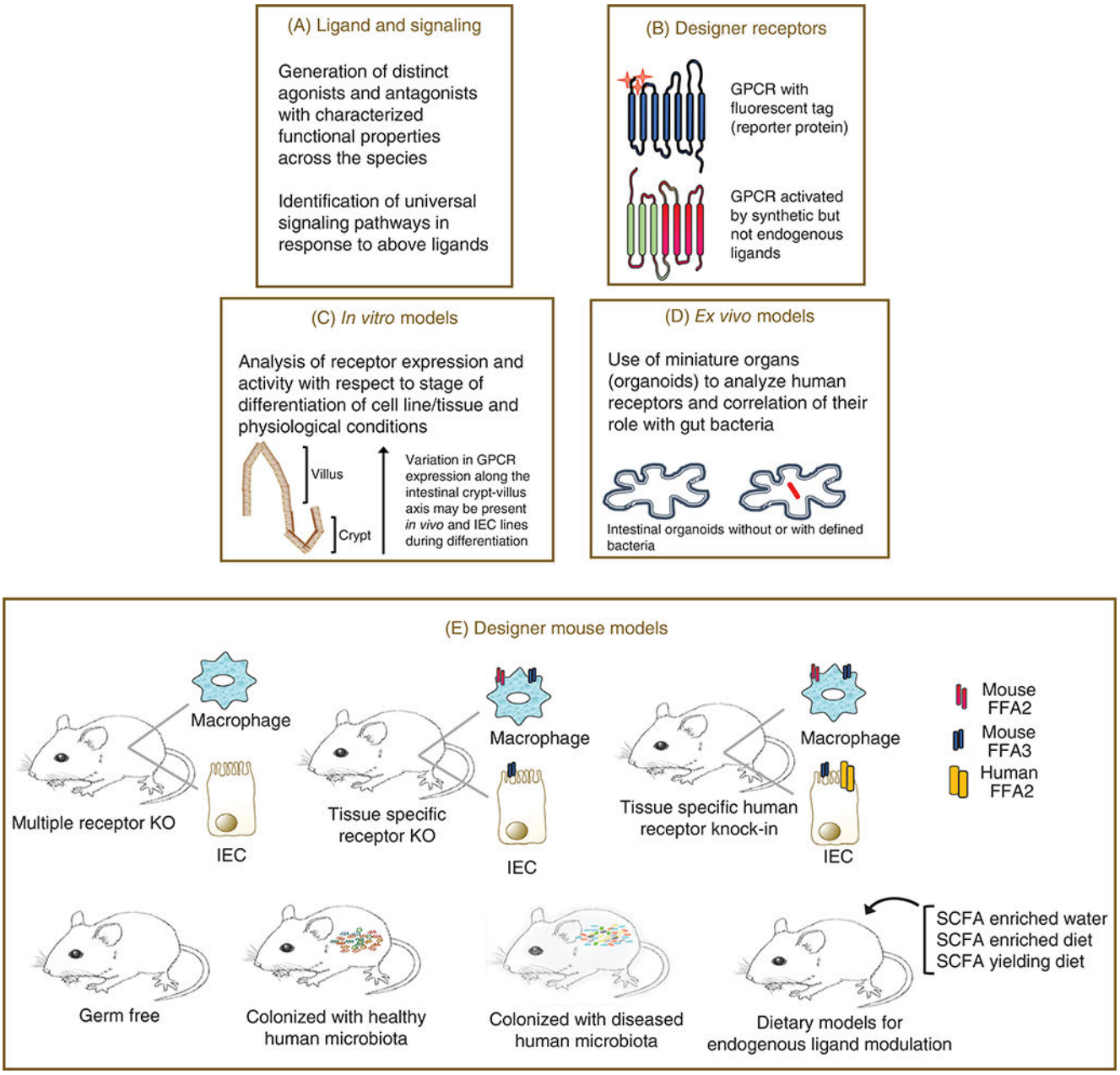

Figure 6.

Customizing our approach for resolution of the roles of short chain fatty acid receptors. Evidence connects short chain fatty acid receptors to gut inflammatory responses. For reasons like shared endogenous ligands, differences in ligand efficacy for species orthologs (and others, enumerated in the text) there are hurdles in assigning selective physiological roles to these receptors. Their actual therapeutic potential thus remains unappreciated. These blocks can be overcome by customized approaches like, development of selective and potent agonists and antagonists for these receptors and their use in *in vitro* and *ex vivo* models (A and D); use of designer receptors with modified ligand binging sites, allowing activation of a particular member from the family which also contains a fluorescent tag (if attached to the

receptor) (B); minimizing discrepancies by determining receptor expression along the course of cell/tissue differentiation (C); several different mouse models can be used to ascertain specific physiological effects of these receptors (E), including use of mice lacking two or more members of the family (multiple receptor KO), tissue specific KO or mice with gene of a human receptor replacing the mouse ortholog (tissue specific human receptor knock-in). These mouse models can then be used in combination with gut microbiota knockdown approaches (germ free), colonized with human microbiota, or fed SCFAs alone or in combination to determine the interrelationship of these receptors with gut microbiota (44,73,94,120,122,123,132,206,218).

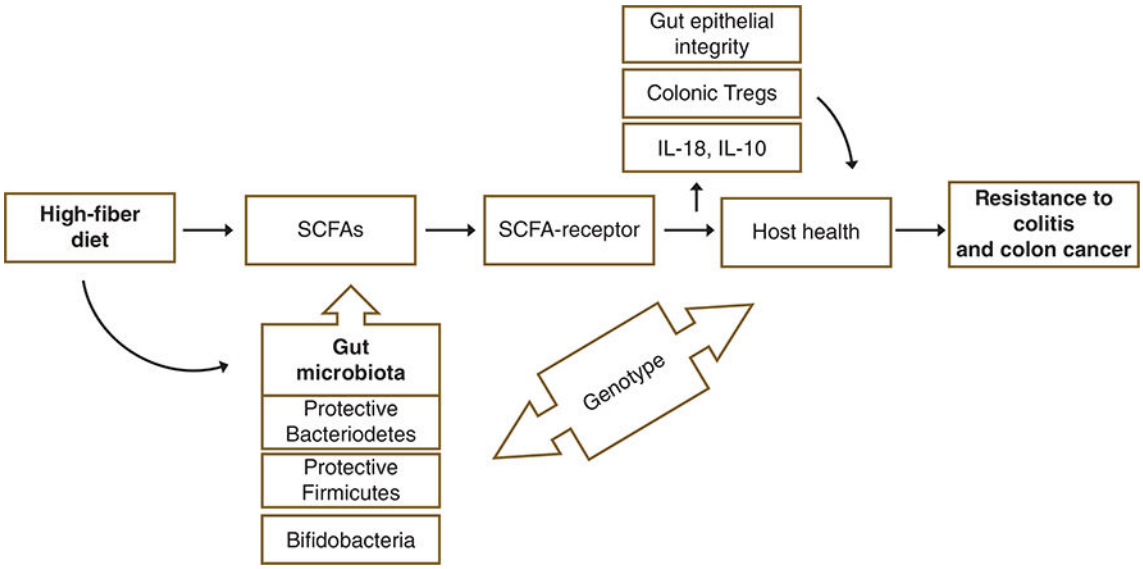

Figure 7.

Interplay of diet, gut microbiota, and short chain fatty acid receptors. Gut microbiota converts fiber in the diet to SCFAs. Activity of SCFAs at their receptors on gut cells promotes epithelial integrity and immune homeostasis. High-fiber diet also shapes and selects for beneficial gut microbiota, and this selection is dependent on the genotype of the host. The combinatorial effect of these processes is resistance against inflammatory states like colitis and colitis associated cancer (111,116,181,190,191).

Table 1

Classification of Nutrient Sensing GPCRs by Nutrient Type

Nutrient	Cognate GPCR
Fatty acids (long)	GPR120, GPR40
Fatty acids (medium)	GPR120, GPR109b, GPR84, GPR40
Fatty acids (short)	FFA2, FFA3, GPR109a, OLFR78
Amino acids	GCN2, T1R1 and T1R3, GRM5 GPRC6A, GPR109b
Carbohydrates	T1R2 and T1R3

Table 2

Short Chain Fatty Acid Receptors: Official and Alternative Names

GPCR (official IUPHAR symbol)	Alternative symbol	Gene	Name
FFA2	GPR43, FFAR2	Ffar2	Free fatty acid receptor 2
FFA3	GPR41, FFAR2	Ffar3	Free fatty acid receptor 3
GPR109a	HCA2, NIACR1, HM74A, PUMA-G	Hcar2	Hydroxycarboxylic acid receptor 2
Olfr78	PSGR; RA1c; MOL2.3; Or51e2; MOR18–2	Olfr78	Olfactory receptor 78

Table 3

Potency (EC50) of Short Chain Fatty Acids at Cognate Receptors

GPCR	Acetate	Propionate	Butyrate
FFA2	35–431	14–290	28–371
FFA3	>1000	6–127	42–158
GPR109a	Does not respond	Does not respond	700–1600
Olfr78	2350	920	NA

All values (in μM) are for human receptors except for Olfr78 where the values are for mouse ortholog (97,100,130,147).