Review

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Future Medicinal Chemistry

Fatty acid and mineral receptors as drug targets for gastrointestinal disorders

Nutrient-sensing receptors, including fatty acid receptors (FFA1–FFA4), Ca^{2+}-sensing receptors and Zn^{2+}-sensing receptors, are involved in several biological processes. These receptors are abundantly expressed in the GI tract, where they have been shown to play crucial roles in regulating GI function. This review provides an overview of the GI functions of fatty acid and mineral receptors, including the regulation of gastric and enteroendocrine functions, GI motility, ion transport and cell growth. Recently, several lines of evidence have implicated these receptors as promising therapeutic targets for the treatment of GI disorders, for example, inflammatory bowel disease, colorectal cancer, metabolic syndrome and diarrheal diseases. A future perspective on drug discovery research targeting these receptors is discussed.

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Intestinal epithelia provide a barrier between the internal components and the external milieus of the body [1]. Gut lumen contains several types of micro-organisms and chemical substances, especially nutrients, including fatty acids (FAs) and minerals, for example, calcium (Ca^{2+}) and zinc (Zn^{2+}). In the past few decades, several types of luminal-facing receptors that are responsive to nutrients (so-called nutrient-sensing receptors), including fatty acid receptors (FFA) and mineral receptors have been identified in intestinal epithelial cells (IEC) [1–4]. Most of these receptors are G-protein-coupled receptors (GPCR), which can be classified into class A or class C GPCRs. For example, short-chain fatty acid (SCFA) receptors belong to the class A GPCR [5], which has seven transmembrane domains, whereas calcium-sensing receptors (CaSR) are classified as a class C GPCR [6], which contains seven transmembrane domains coupled with the large extracellular domain. Topological illustrations of these nutrient-sensing GPCRs are shown in Figure 1.

In general, nutrient-sensing GPCRs are coupled with various types of Gα subunits including $G\alpha_s$, $G\alpha_q$, $G\alpha_i$, $G\alpha_{12/13}$ and gustducin. The physiological effects produced by these receptors in response to nutrients are mainly mediated via cAMP and Ca^{2+} signaling cascades [1]. Furthermore, the membrane localization and signaling of nutrient-sensing receptors is regulated by β-arrestin. After receptor activation, β-arrestin is recruited to the ligand-bound receptors, which enables receptor internalization and degradation leading to receptor desensitization [7]. Furthermore, it has recently been shown that β-arrestin forms a complex with activated GPCR, which results in sustaining intracellular signaling [8]. In addition to β-arrestin, nutrient-sensing GPCR can be regulated by GPCR kinase (GRK) [9,10]. Phosphorylation of nutrient-sensing GPCRs by GRK results in an increased binding affinity between β-arrestin and the receptors, which in turn leads to an enhanced receptor internalization and refractory period of the cells [11]. A comprehensive view of the regulation of

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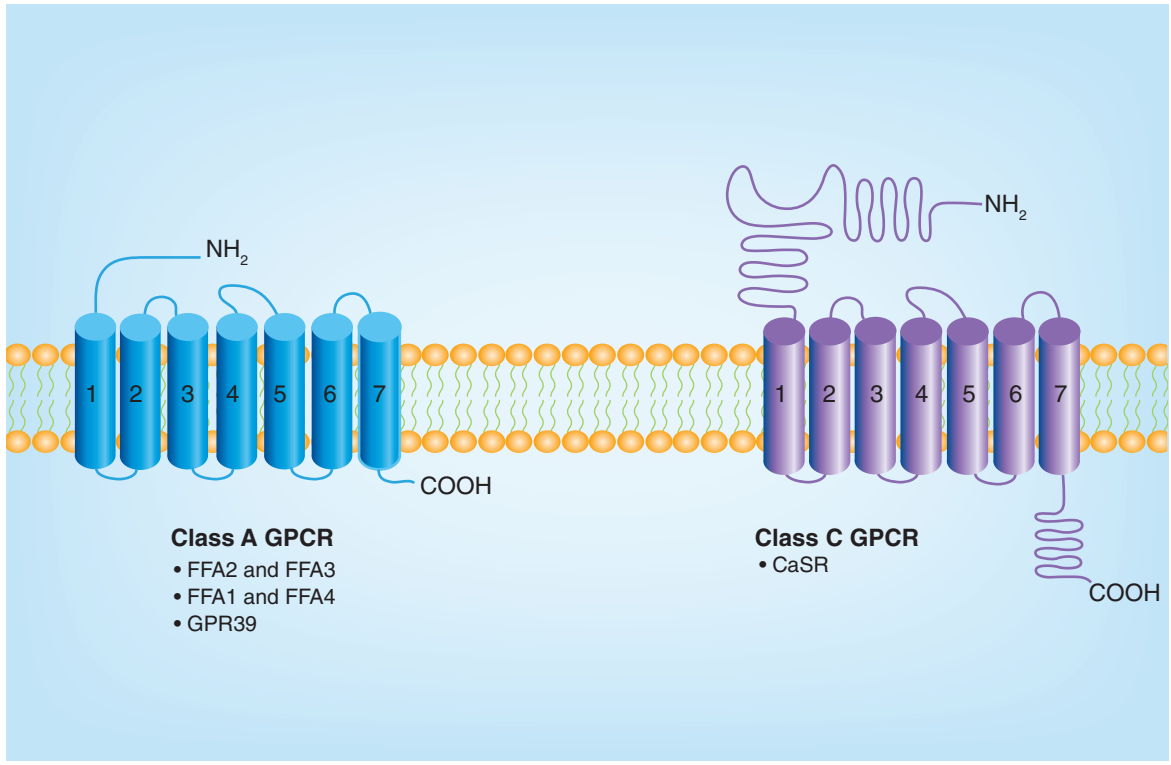

Figure 1. Structures of class A and class C nutrient-sensing G-protein-coupled receptor. Fatty acid receptors, including FFA1–FFA4 and zinc-sensing receptor (GPR39), are included into the class A GPCRs, which contain seven transmembrane proteins. Calcium-sensing receptor belongs to class C GPCRs, which comprise seven transmembrane proteins containing a large extracellular domain.
CaSR: Calcium-sensing receptor; GPCR: G-protein-coupled receptor.

nutrient-sensing GPCRs by β-arrestin and GRK is illustrated in Figure 2. This review summarizes the current state of knowledge regarding the physiological functions and implications of the nutrient-sensing receptors, especially FFA and mineral receptors, as drug targets for gastrointestinal (GI) disorders.

Fatty acid receptors

Short-chain fatty acid receptors FFA2 & FFA3

Background on SCFA receptors

Among the deorphanized GPCRs, GPR41 and GPR43 have been identified to be predominantly agonized by SCFAs, which are the saturated organic carboxylic acids containing 2–6 carbon atoms (C2-C6) [12]. Because of this finding, GPR41 and GPR43 are included in the family of FFA and assigned as FFA3 and FFA2, respectively [5]. FFA2 and FFA3, together with the medium- and long-chain fatty acid (MCFA and LCFA) receptors FFA1 and FFA4, belong to the class A GPCRs, which comprise seven transmembrane domains [5]. Although localization profile of these SCFA receptors is not clearly identified yet, luminal SCFAs mediate activation of these receptors [13], suggesting that both FFA2 and FFA3 may partly express in apical membrane of IEC. FFA2 and FFA3 share ~43% similarity in their amino acid sequences [5]. Within the conserved amino acid residues of both FFA2 and FFA3, Arg^{180} and Arg^{255} as well as His^{242} are important for ligand recognition and signal transduction. The mutagenesis of these residues diminishes stimulation of the agonist-induced FFA2 and FFA3 [14]. These receptors are stimulated by acetate (C2 SCFA), propionate (C3 SCFA) and butyrate (C4 SCFA), which are the major endogenous SCFAs primarily fermented from indigestible carbohydrates by anaerobic bacteria in the intestine [1,5]. Interestingly, these SCFAs bind differentially to FFA2 and FFA3. FFA2 is more specific to C2-C3 SCFAs than C4-C5 SCFAs, whereas FFA3 preferentially recognizes C3-C5 SCFAs rather than C2 SCFAs [15]. Based on this information, acetate (C2 SCFA) and butyrate (C4 SCFA) are used as specific agonists of FFA2 and FFA3, respectively [12,15]. It is well accepted that carboxylic group is the key element of SCFAs that activate FFA2 and FFA3. Structure–activity relationship analysis and mutagenesis and molecular docking studies have revealed that the small carboxylic acid compounds containing sp^2- or sp-hybridized α-carbon selectively stimulate FFA2 over FFA3, whereas small carboxylic acid containing sp^3-hybridized α-carbon

specifically agonizes FFA3 at the orthosteric binding site [15]. In addition, highly selective FFA2 and FFA3 agonists and antagonists have been identified and used to delineate the physiological functions of these two receptors [16]. The chemical structures of the specific agonists and antagonists of FFA2 and FFA3 are shown in Figure 3. Indeed, 4-chloro-α-(1-methylethyl)-*N*-2 thiazolylbenzeneacetamide (4-CMTB) and the *N*-(2,5-dichlorophenyl)-4-(furan-2-yl)-2-methyl-5-oxo-1,4,5,6,7,8-hexahydro-quinoline-3 carboxamide (AR420626) are recognized as the specific agonists of FFA2 and FFA3, respectively, which potently activate these receptors with EC_{50} in the range of >200 nM to 10 μM [17,18]. GLPG0974 antagonized SCFA-induced FFA2 activation with an IC_{50} in nanomolar ranges [19].

Indeed, the signal transduction pathways of FFA2 and FFA3 are different. FFA2 is capable of transducing the signal through $G\alpha_q$ and $G\alpha_i$, leading to stimulation of ERK [20]. Moreover, FFA2 agonism is accompanied by β-arrestin recruitment, causing internalization of FFA2 [11]. FFA3 signaling is mediated by $G\alpha_i$ and gustducin, a G protein associated with taste perception [21], which subsequently induces a reduction of intracellular cAMP and cGMP levels [22].

Since their identification, FFA2 and FFA3 have been studied for their physiological and pharmacological roles in various cells and tissues, including immune cells, neurons, pancreas and adipose tissues [16]. In particular, because SCFAs are produced in the intestine where they are present in high concentrations [23], the roles of FFA2 and FFA3 in regulating GI functions and in modulating the pathogenesis of GI disorders have been actively investigated (Figure 4).

Physiological roles of FFA2 & FFA3 in the GI tract

Regulation of intestinal immune defense by FFA2 & FFA3

As a part of innate immune defense, IEC forms a physical barrier that prevents the invasion of pathogens into our bodies [24]. Following bacterial invasion, chemotaxis occurs and innate immune cells especially neutrophils are recruited to the infected area to eradicate the invading pathogens [25]. In addition, effector T cells, which constitute an adaptive immune system, contribute to the process of bacterial clearance, especially during chronic infection [26].

Butyrate, an endogenous ligand of both FFA2 and FFA3, has been shown to induce tight junction assem-

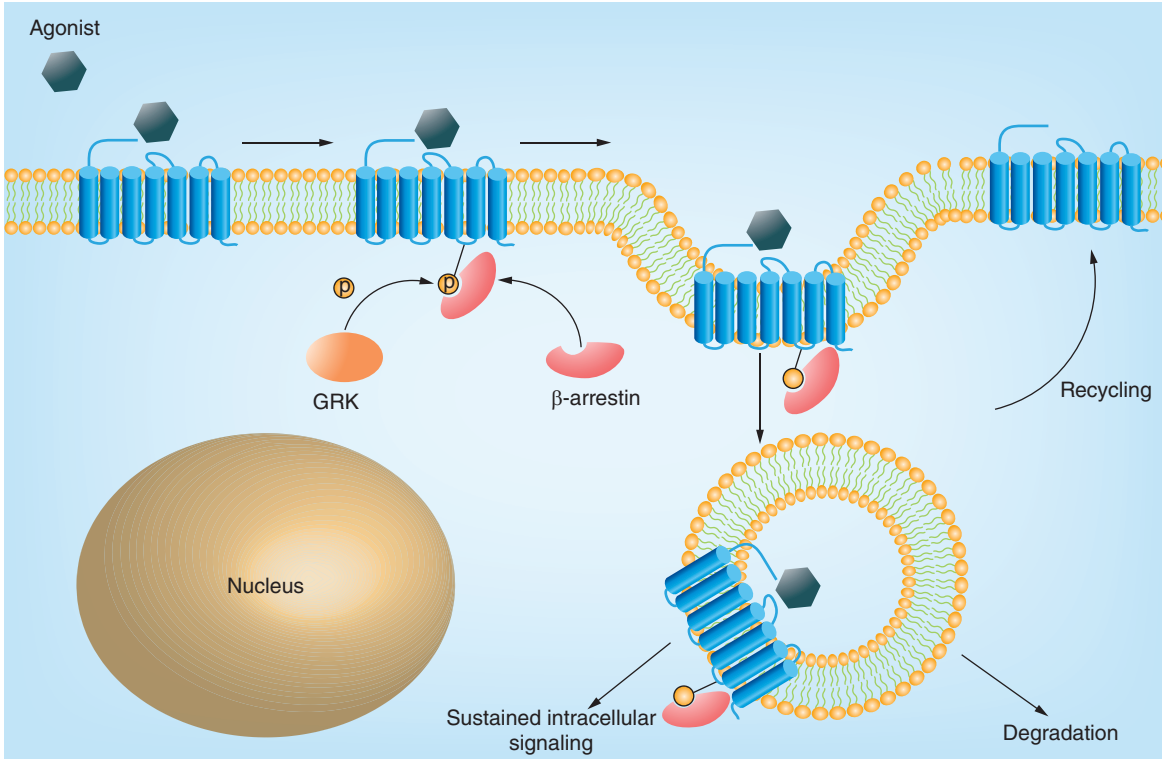

Figure 2. Regulation of signal transduction through nutrient-sensing G-protein-coupled receptors. The membrane expression of nutrient-sensing G-protein-coupled receptors (GPCRs) is determined by β-arrestin and GRK. β-arrestin induces the internalization and degradation of nutrient-sensing GPCRs. Alternatively, it can sustain intracellular signaling through GPCRs. GRK plays a key role in sustaining an interaction between GPCR and β-arrestin.
GRK: GPCR kinase.

bly and protect against ethanol-induced intestinal barrier dysfunction in Caco-2 cells via activation of AMPK, an intracellular energy sensor known to promote tight junction assembly [27]. Through the use of transgenic mice expressing a monomeric red fluorescent protein (mRFP) reporter, the expression of FFA2-mRFP was found to be mainly localized in leukocytes in the lamina propria of the intestine [16,17]. The mechanisms of FFA2-induced neutrophil chemotaxis involve the activation of several signaling molecules including

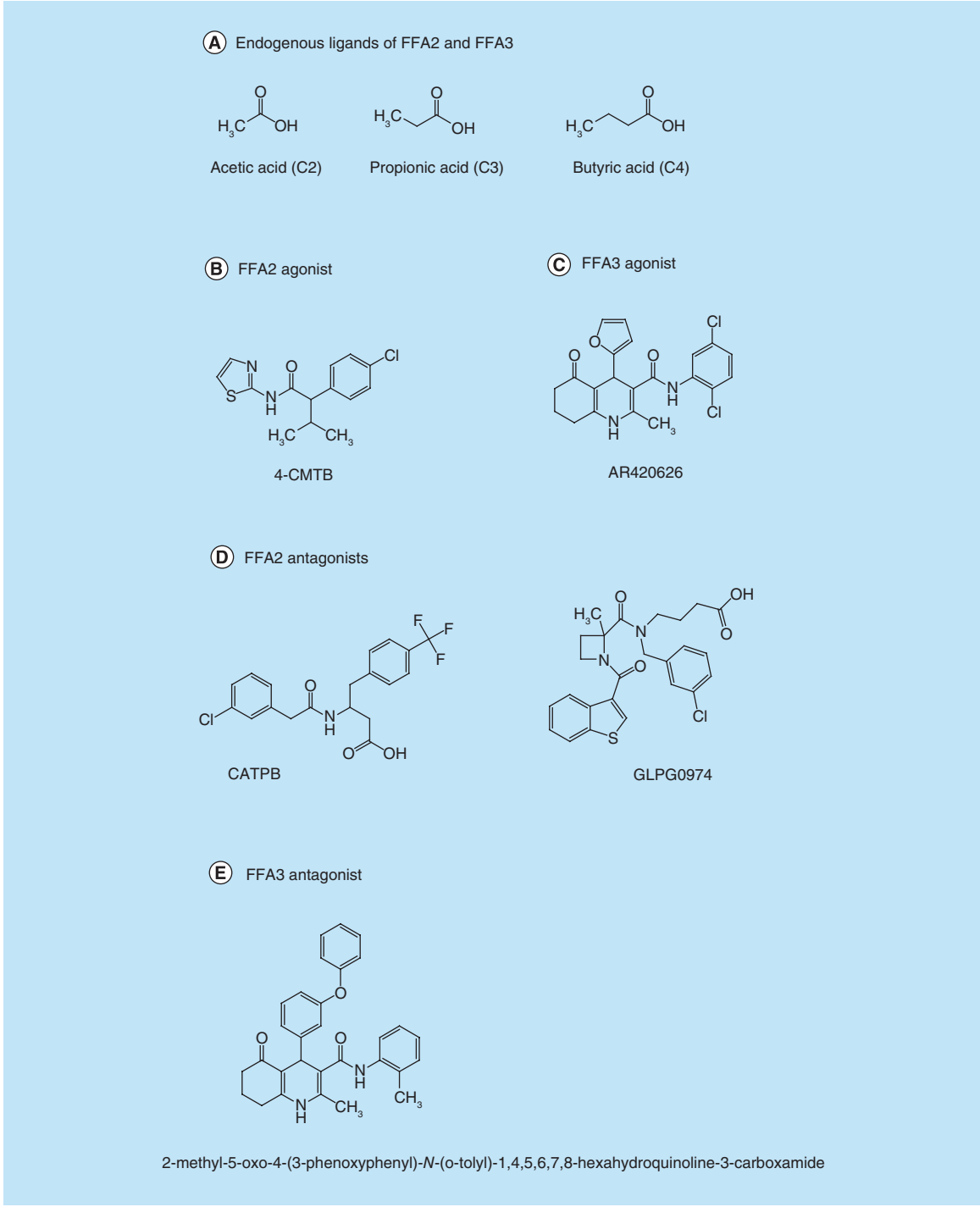

Figure 3. Chemical structures of short-chain fatty acid receptor agonists and antagonists. (A) Representative endogenous ligands of FFA2 and FFA3. **(B)** Synthetic agonist of FFA2. **(C)** Synthetic agonist of FFA3. **(D)** Synthetic antagonists of FFA2. **(E)** Synthetic antagonists of FFA3.
FFA: Fatty acid receptor; SCFA: Short-chain fatty acid.

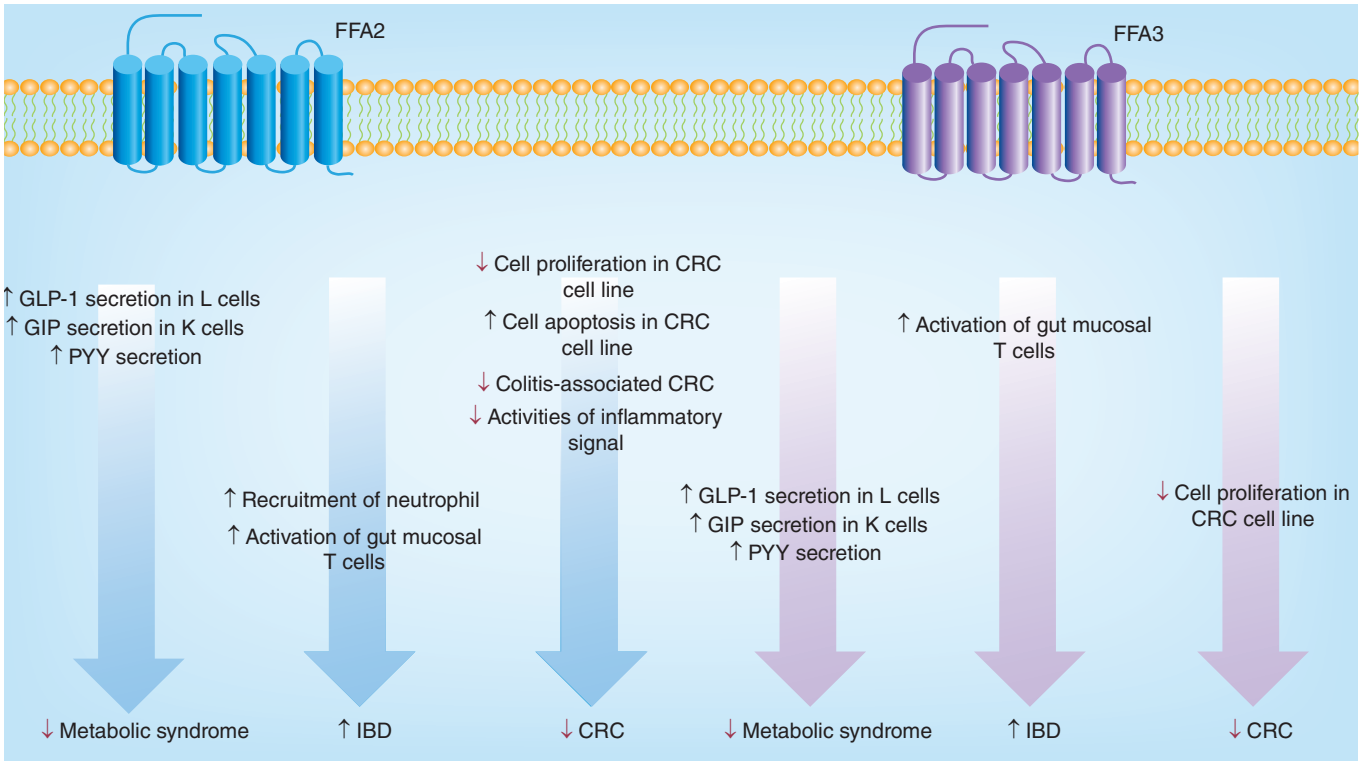

Figure 4. Roles of short-chain fatty acid receptors in the regulation of GI functions and as drug targets for GI disorders. Activation of FFA2 and FFA3 results in the release of gut hormones, including GLP-1, GIP and PYY, which are the target for the treatment of metabolic syndrome. In addition, these receptors also induce intestinal immune responses and suppress intestinal cell proliferation. Therefore, these receptors have been proposed to be therapeutic targets for colitis and CRC.
CRC: Colorectal cancer; GIP: Glucose-dependent insulinotropic polypeptide; GLP-1: Glucagon-like peptide-1; IBD: Inflammatory bowel disease; PYY: Peptide tyrosine tyrosine.

p38 MAPK, PKB, PI3Kγ, Rac2 and ERK [12,20,28]. In addition, it has previously been found that both FFA2 and FFA3 are essential to elicit an intestinal immune response against *Citrobacter rodentium* infection through a mechanism involving stimulation of effector T cells [29]. Moreover, FFA2 and FFA3 KO mice exhibited reduced adaptive immune response and bacterial clearance capacities in the intestine [29]. Indeed, the mechanisms underlying FFA2- and FFA3-mediated effector T-cell activation in intestinal mucosa has been shown to mediate via ERK and MAPK signaling [29]. Therefore, FFA2 and FFA3 appear to play important roles in boosting intestinal immune defense against enteric infection.

FFA2 & FFA3 are the regulators of gut hormone secretion

In addition to serving digestive and absorptive functions, the GI tract helps maintain energy homeostasis in the body through the effects of gut hormones. Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (also called gastric inhibitory polypeptide or GIP) are released from L cells and K cells, respectively. These two incretin hormones potentiate glucose-stimulated insulin secretion (GSIS), which is also known as an insulinotropic effect [30]. The appetite-suppressing hormones, including cholecystokinin (CCK) and peptide tyrosine tyrosine (PYY), are also released from the enteroendocrine cells of the intestine [31]. Interestingly, gut microbiota [32] and SCFAs [33] enhance the rate of gut hormone releases, raising the possibility that SCFA receptors may control body metabolism in part by regulating gut hormone secretion.

Using quantitative PCR and transgenic mice with an mRFP reporter, FFA2 and FFA3 expressions are mainly found in GLP-1-secreting L cells. Furthermore, FFA3-mRFP expression, but not FFA2-mRFP expression, was predominantly detected in CCK-, GIP- and PYY-secreting cells [17]. Consistent with the expression profile, SCFA-mediated GLP-1 secretion was significantly reduced in mice lacking FFA2 and FFA3 [34]. Likewise, synthetic agonists of FFA2 and FFA3 induced GLP-1 secretion in cultured colonic crypts and in animal models [17,35]. The mechanism of GLP-1 secretion induced by the stimulation of these receptors involves Ca^{2+}- and α-gustducin-dependent signaling pathways [12,17,35]. Apart from this, agonism of FFA2 and FFA3 triggers

PYY [36] and GIP secretion [36]. All of these data indicate that FFA2 and FFA3 stimulation in enteroendocrine cells induces GLP-1 and GIP release, which may potentiate insulin secretion from pancreatic β cells, and induces PYY secretion, which may reduce appetite. Future investigations should be emphasized to determine the beneficial effects of FFA2 and FFA3 agonism in the treatment of obesity and diabetes.

Roles of FFA2 & FFA3 as drug targets

FFA2 & FFA3 as drug targets for inflammatory bowel disease

In inflammatory bowel disease (IBD), including Crohn's disease and ulcerative colitis, a defective intestinal barrier function and dysregulation of mucosal immunity leads to chronic intestinal inflammation and associated mucosal damages [37]. TNF-α, a cytokine released from immune cells, especially neutrophils, has been implicated in the pathogenesis of IBD in part by inducing IEC apoptosis, which results in intestinal barrier disruption and submucosal immune activation. Indeed, anti-TNF-α antibody is considered a drug candidate for the treatment of IBD [38].

Acetate, an FFA2 agonist, has been found to suppress inflammatory responses and ameliorate the severity of colitis by reducing TNF-α expression. Intestinal inflammation was also exaggerated in FFA2 KO mice [39,40]. Of note, anti-FFA2 antibodies abolished the inhibitory effect of acetate on intestinal inflammation in dextran sulfate sodium (DSS)-induced colitis in mice [39]. Interestingly, an FFA2 agonist has been shown to protect against DSS-induced colitis via mechanisms involving K^+ efflux-induced hyperpolarization leading to activation of the NLRP3 inflammasome and caspase-1 in colonic epithelial cells, including HT-29 and NMC460 cells. These mediators induced IL-18 release from mouse colon in a DSS-induced colitis model, which subsequently promoted intestinal epithelial repair [41,42]. Furthermore, FFA2 and FFA3 are localized in L cells and activation of these receptors is associated with an increase in concentrations of glucagon-like peptide-2 (GLP-2) in the portal blood [43]. Since GLP-2 has pro-survial, anti-inflammatory and barrier strengthening effects [44], activation of SCFA receptors may be of therapeutic value in the treatment of IBD and short bowel syndrome via the effect of released GLP-2 [45]. By contrast, other research groups have reported conflicting evidence that these receptors mediate intestinal inflammation by eliciting chronic immune response in a mouse model of colitis [28,29]. In support of this statement, dietary fiber intake has been shown to be strongly associated with the severity of active Crohn's disease concurrent with an increase in infiltration of FFA2-positive immune cells [46]. This observation suggests that the SCFA derived from the fiber might promote the occurrence of colitis and the roles of FFA2 and FFA3 in enhancing inflammation in IBD might be envisioned. In agreement with this notion, FFA3 KO mice showed a reduction of neutrophil recruitment and intestinal destruction in a chronic colitis model [28]. In addition, FFA2 and FFA3 have been shown to induce immune responses in certain circumstances, such as during bacterial infection [28,29]. Importantly, GLPG0974, an FFA2 antagonist, has been shown to suppress acetate-induced neutrophil activation [19,47], suggesting that inhibition of FFA2 may be useful in the treatment of IBD. Currently, GLPG0974 is in Phase I clinical trials, and it has been proposed as a drug candidate for treating patients with mild to moderate ulcerative colitis [19,47]. Based on the contradictory roles of SCFA receptors in IBD, roles of these receptors in the regulation of intestinal immune responses and intestinal barrier integrity and in IBD pathogenesis warrant further investigations. Moreover, FFA2 and FFA3 are highly expressed in duodenal enterochromaffin cells and activation of these receptors stimulates serotonin (5-HT) release, which subsequently produces peristaltic effects [43,48]. Therefore, inhibition of SCFA receptors may also be beneficial in the treatment of irritable bowel syndrome.

SCFA receptors & colorectal cancer

The pathogenesis of colorectal cancer (CRC) involves chronic inflammation and overactivation of Wnt signaling [49]. The NF-κB signaling pathway represents the major inflammatory pathway involved in CRC pathogenesis through the effects of downstream inflammatory mediators, including COX-2, IL-1β and TNF-α [50]. The longstanding inflammation promotes the accumulation of gene mutations, which leads to the development of CRC.

Several lines of evidence have shown that FFA2 serves as a drug target for the treatment of CRC. For example, FFA2 expression is downregulated in human colon cancer cell lines [51]. The overexpression of FFA2 in HCT8 cells, an ileocecal colorectal adenocarcinoma cell line, results in cell cycle arrest and increases cell apoptosis by downregulating cyclin D3 and cyclin-dependent kinases, which are positive regulators of cell cycle progression, concurrent with an increase in expression of p21, an inhibitor of cyclin-dependent kinases [51]. In the colitis-associated CRC model, FFA2 KO mice are more susceptible to the induction of tumorigenesis by the combined treatment of azoxymethane and DSS compared with the wild-type mice [52]. Similarly, using a rat model of inflammation-associated CRC, administration of dietary resistant starch led to an increase in SCFA production, a decrease in expression of inflammatory media-

tors, including NF-κB, COX-2, IL-1β and TNF-α transcripts, and a decrease in intestinal cell proliferation [53]. Furthermore, butyrate, an FFA3 agonist, has been shown to exert a chemopreventive effect in CRC models via suppression of Wnt signaling [54]. Therefore, stimulation of FFA2 and FFA3 may be beneficial in the treatment of CRC. However, further studies are needed to provide a proof-of-concept that FFA2 and FFA2 are drug targets of CRC.

Polyunsaturated fatty acid receptors FFA1 & FFA4

Background on FFA1 & FFA4

FFA1 and FFA4, also known as GPR40 and GPR120, respectively, are specifically agonized by MCFAs and LCFAs, including essential polyunsaturated fatty acids (PUFA) belonging to the Ω-3 family, for example, α-linolenic acid, eicosapentaenoic acid and docosahexaenoic acid [5,55]. Although their phylogeneticity is different [56], FFA1 and FFA4 are activated by similar endogenous agonists. Structurally, arginine residues located in the transmembrane regions 5 (Arg^{183}) and 7 (Arg^{258}) of FFA1 [57,58] as well as in the transmembrane region 2 (Arg^{99}) of FFA4 [59] play critical roles in determining the suitable conformation required for binding with carboxylic groups of FA. Moreover, the structure–activity relationship analysis of FFA4 agonists suggested that negative charge was required for FFA4 activation, and it was also determined by substituents in biphenyl part of hydrophobic tail [59]. Intensive information on ligand-receptor binding has previously been reviewed [59–61]. Within the past decade, several agonists and antagonists of FFA1 and FFA4 have been identified. The chemical structures of these compounds are shown in Figure 5. They have EC_{50} values for Ca^{2+} mobilization in low micromolar ranges (≤10 μM) [9].

It has been shown that FFA1 and FFA4 stimulation causes ERK phosphorylation via a $G\alpha_q$-Ca^{2+} signaling pathway [62–65]. In addition, these receptors can also bind with and trigger β-arrestin signaling [9]. In addition, it has been shown that the recruitment of β-arrestin by an FFA1 agonist TAK-875 potentiated the insulinotropic effect in β cells [66], followed by β-arrestin-mediated internalization and the recycling of FFA1 [67]. In contrast, internalized FFA4 following β-arrestin recruitment was committed to lysosomal degradation rather than recycling [68]. Other G proteins, such as $G\alpha_s$ and $G\alpha_i$, can also mediate the signal transduction of FFA1 [69] and FFA4 [17,70]. Indeed, these GPCRs have been demonstrated to be phosphorylated by GRK, resulting in the modulation of their signal transduction [10]. For example, phosphorylation of FFA4 by GRK led to increased binding affinity between FFA4 and β-arrestin, which facilitated FFA4 internalization and desensitization [9].

FFA1 and FFA4 are ubiquitously expressed in the epithelial cells and enteroendocrine cells of the GI tract [71,72]. Along the length of GI tract, overall expression of FFA4 is increased from small to large intestine [71], whereas the information of FFA1 expression in each GI tissues is elusive. Expression of FFA1 and FFA4 is relatively more abundant in enteroendocrine cells than in other cell types in the gut mucosa [72–75], suggesting the important role of these receptors in gut hormonal regulation. It was believed that FFA1 is located on the basolateral side [76].

Herein, we discuss the roles of FFA1 and FFA4 in the regulation of GI functions and as drug targets for GI disorders. An illustration of the GI functions and GI diseases mediated through these receptors is shown in Figure 6.

Physiological roles of FFA1 & FFA4

Function of FFA1 & FFA4 in enteroendocrine cells

Accumulated lines of evidence have revealed the roles of MCFAs and LCFAs in controlling the secretion of incretin hormones [77]. Using fluorescence-activated cell sorting, FFA1 mRNA was found to be plentiful in L cells [78] and K cells [79]. The functional roles of these receptors in enteroendocrine cells have been reported. GW9508, a FFA1/FFA4 agonist, promoted GLP-1 secretion from human colonic primary cells [80]. FFA1 also mediated the FA-induced GLP-1 secretion from mouse L cells [72] and suppressed food intake [81]. Similarly, Luo *et al.* found that an FFA1 agonist AM-1638 increased GLP-1 and GIP secretion in a mouse model of high-fat diet (HFD)/streptozotocin-induced metabolic syndrome [82]. Interestingly, in a rat intestinal perfusion model, intravascular administration of various types of FFA1 synthetic agonists significantly promoted GLP-1 secretion, whereas intraluminal injections of these agonists had no effect [76]. These findings implied that the effect of FFA1 agonists on inducing GLP-1 and GIP secretion requires intestinal absorption. Similar to FFA1, FFA4 is expressed in the ileum and colon of mice and humans, especially in L cells and K cells [78,79]. FFA4 agonists induced an increase in GLP-1 secretion from human primary enteroendocrine cells [80]. Using both genetic and pharmacological manipulations, it has been demonstrated that FFA4 is required for FA-induced GLP-1 and GIP secretion [73]. Taken together, FFA1 and FFA4 act as fat sensors, for which activation leads to incretin secretion in both L cells and K cells.

After a caloric meal, duodenal I cells release CCK, which is involved in the digestion of protein and fat.

Notably, I cells express FFA1 and FFA4 [74,83]. However, the physiological role of LCFA receptors in I cells is still elusive. In fact, the elevation of plasma CCK in response to unsaturated oil has been found to be markedly reduced in FFA1 KO mice [74]. Interestingly, Tanaka and coworkers demonstrated that LCFA-induced CCK secretion in STC-1 cells, which are enteroendocrine cell lines, is mediated by FFA4 but not FFA1 through a mechanism involving extracellular Ca^{2+} influx via L-type Ca^{2+} channels [83]. Moreover, FFA4 has recently been found to regulate gastric somatostatin and ghrelin secretion from D

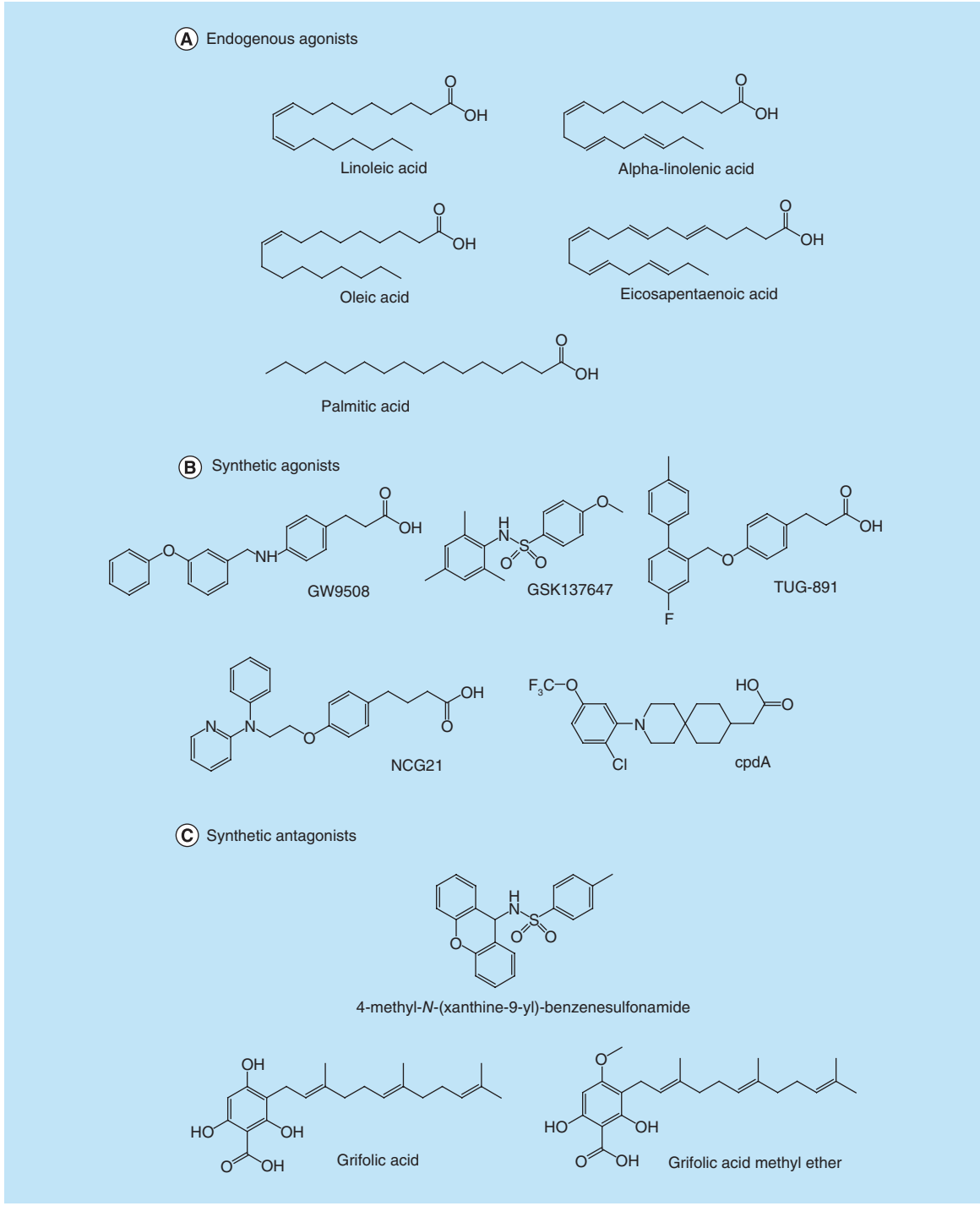

Figure 5. Chemical structures of long-chain fatty acid receptor agonists and antagonists. (A) Examples of endogenous ligands of FFA1 and FFA4. **(B)** Synthetic agonists of FFA1 and FFA4. **(C)** Synthetic agonists of FFA1 and FFA4.

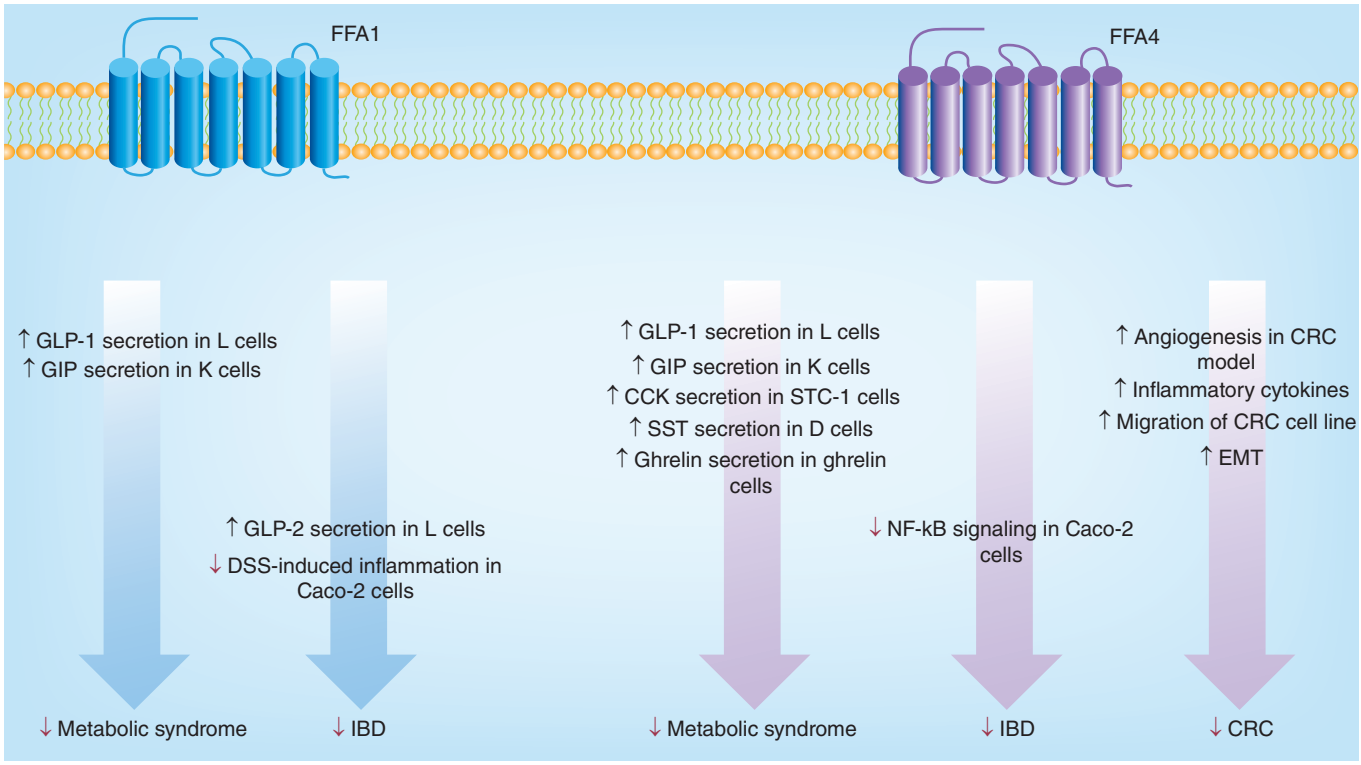

Figure 6. Physiological and pathological roles of long-chain fatty acid receptors in the GI system. Roles of FFA1 and FFA4 in the regulation of GI function and as drug targets for GI diseases. FFA1 and FFA4 enhanced the secretion of incretins, which are a target for an antiobesity. Activation of FFA1 increased GLP-2 release, while FFA4 decreased the secretion of GLP-2, whereas, these receptors inhibited inflammatory signaling in IEC. Thus, an agonist of FFA1 may be beneficial in the treatment of IBD. In addition, FFA4 elevated the hallmarks of CRC progression. FFA4 antagonist may be a promising treatment for CRC.
CCK: Cholecystokinin; CRC: Colorectal cancer; DSS: Dextran sulfate sodium; EMT: Epithelial–mesenchymal transition; FFA: Fatty acid receptor; IBD: Inflammatory bowel disease; SST: Somatostatin.

cells and ghrelin cells, respectively. These two hormones maintain gastric secretion, gastric motility and feeding behavior [84]. Interestingly, a synthetic agonist of FFA4 abrogated somatostatin [85] and ghrelin secretion from these cells [17].

In summary, FFA1 and FFA4 in enteroendocrine cells may serve as sensors of ingested LCFA, which regulate the secretion of various gut hormones involved in maintaining digestion, appetite and energy homeostasis.

Roles of FFA1 & FFA4 as drug targets for GI diseases

FFA1 & FFA4 as drug targets for metabolic syndrome

One of the GI functions related to metabolic syndrome is the secretion of incretin hormones from enteroendocrine cells [30]. As previously discussed, FFA1 and FFA4 are the positive regulators of incretin secretion, which is known to be beneficial in the treatment of Type 2 DM (T2DM) [30,77]. Indeed, expression of FFA1 and FFA4 have been reported to be altered in several models of metabolic syndrome. For example, FFA1 expression was increased in the intestinal mucosa of HFD-fed mice and rats [86]. High glucose and FA levels may have induced the FFA1 expression in these models [72,87]. Treatment with FFA1 agonists enhanced glucose-stimulated insulin secretion in a T2DM mouse model by promoting incretin secretion [82]. Increased FFA4 expression was also found in the gastric mucosa and duodenal mucosa of HFD-fed mice and obese subjects [88]. In obese people, BMI was positively correlated with duodenal FFA4 expression and inversely correlated with the number of enteroendocrine cells. However, conflicting results have been reported. Expression of FFA4 in ileal tissues and the number of L cells were reduced in diet-induced obese models [89,90]. Intriguingly, FFA4 KO mice and humans with the FFA4 R270H variant, a FFA4 mutant with impaired receptor activity, have been found to be more susceptible to HFD-induced obesity compared with wild-type/normal subjects [91,92]. Based on the current state of knowledge, it is speculated that FFA4 agonists may be beneficial in the treatment of obesity and metabolic syndrome.

FFA1 & FFA4 as drug targets for IBD

As Ω-3 PUFAs are known for their anti-inflammatory effect [93], the roles of FFA1 and FFA4 in modulating inflammation have extensively been investigated in several organs and cell types, such as adipose tissues, macrophages and skeletal muscle [9]. Particularly, FFA1 and FFA4 are expressed in the intestinal tissues of mice and rats [73,94] and in human IEC [62,95]. Activation of FFA1 in IEC by 10-hydroxy-cis-12-octadecenoic acid, a gut microbial metabolite of linoleic acid, attenuated DSS-induced and inflammatory cytokine-induced tight junction dysregulation, a hallmark of IBD, in IEC line Caco-2 cells [62]. In addition, supplementation with Ω-3-enriched oil increased FFA4 expression and suppressed expression of TNF-α, a major proinflammatory mediator involved in IBD pathogenesis, in the colonic epithelia of mice [94]. Interestingly, TUG-891, a specific ligand of FFA4, suppressed NF-κB signaling via β-arrestin 2-dependent mechanisms in Caco-2 cells, but not in STC-1 enteroendocrine cells [71]. This finding suggests that the anti-inflammatory effect of FFA4 stimulation is restricted to IEC. In addition, it has recently been shown that FFA1 and FFA4 in L cells play a role in the regulation of intestinal inflammation by inducing the secretion of GLP-2 [95], a local anti-inflammatory hormone derived from enteroendocrine cells [44]. Under physiological conditions, PUFA treatment increases GLP-2 secretion via FFA1–Ca^{2+}–ERK signaling pathways. Under pathological conditions, for example, IBD, TNF-α increases FFA4 expression in the ileal mucosa of IBD patients and in rat L cells. The increased FFA4 expression leads to a reduction in GLP-2 secretion, at least in part, via a cAMP-dependent mechanism [95]. Therefore, these data suggest the contradictory roles of FFA1 and FFA4 on GLP-2 secretion, which may be implicated in the treatment of IBD.

FFA1 & FFA4 as anticolorectal cancer drug targets

Expression of FFA4 but not FFA1 was increased in CRC cell lines and CRC tissues compared with normal cells and tissues [96]. In addition, FFA4 expression was correlated with advanced clinical stages of CRC patients [96]. It has also been shown that FFA4 activation leads to inflammatory cytokine production, angiogenesis and the migration of HCT-116 and SW480 cells, which are the hallmarks of cancer progression. Furthermore, expression of FFA4 was correlated with a reduction of E-cadherin, promoting epithelial–mesenchymal transition (EMT), which is a phenomenon important for the initiation of CRC metastasis [96]. These findings indicate that FFA4 may be involved in CRC development and FFA4 antagonist may be of particular value in CRC treatment. However, future investigations are required to validate these two receptors as anti-CRC drug targets.

Mineral receptors

Calcium-sensing receptor

Background on CaSR

CaSR was initially identified in the bovine parathyroid gland in 1993 [2]. The full length of the human *CaSR* gene was subsequently cloned [97] and identified as a member of the class C GPCR family [6]. CaSR displays pleiotropic interplay among $G\alpha_q$, $G\alpha_i$ and $G\alpha_{12/13}$ in various cell types [98]. Nevertheless, in certain cell types, such as pituitary cells [99] and breast cancer cells [100], CaSR is specifically coupled with $G\alpha_s$. CaSR plays a crucial role in the systemic regulation of Ca^{2+} homeostasis, at least in part, by attenuating the rate of parathyroid hormone secretion [6]. Apart from Ca^{2+}, CaSR can be agonized by other divalent cations (e.g., Mg^{2+} and Sr^{2+}), trivalent cations (La^{3+} and Gd^{3+}) and L-amino acid (L-tryptophan; L-Trp) [101,102]. In addition, the physiological roles of CaSR in other tissues have recently been demonstrated [6]. Indeed, several classes of CaSR agonists and antagonists have been identified and proven useful in revealing the functions of CaSR under both physiological and pathological settings. The chemical structures of CaSR modulators are shown in Figure 7.

CaSR is ubiquitously expressed along the GI tract, especially in the basolateral membrane of epithelial cells [103]. Several lines of evidence have noted the important roles of CaSR in the GI tract [6]. Herein, we provide an overview of the physiological roles of CaSR in the GI tract and discuss the potential roles of CaSR as a therapeutic target for GI disorders (Figure 8).

Physiological roles of CaSR in GI tract

CaSR as a regulator of gastric acid secretion

In the stomach, CaSR is highly expressed in the basolateral membrane of parietal cells [104,105]. CaSR agonism induced gastric acid secretion by enhancing H^+-K^+-ATPase activity [105]. The mechanism of CaSR-induced H^+-K^+-ATPase hyperactivity is partly via the pertussis toxin-sensitive G protein-Ca^{2+}-PKC-ERK-MAPK pathways [106]. In addition, activation of CaSR could indirectly stimulate gastric acid secretion by inducing gastrin secretion [104].

CaSR as a regulator of intestinal cell differentiation, proliferation & apoptosis

It has been reported that CaSR is required for TGF-β-mediated cell differentiation in human colonic epithelial CBS cells which are derived from differentiated human colorectal tumors [107]. Moreover,

CaSR activation in myofibroblasts and IEC caused an induction of Wnt5a secretion and increased membrane expression of the Wnt5a receptor Ror2, respectively [108]. This observation suggests that CaSR is involved in colonic differentiation through a mechanism involving the potentiation of Wnt5a-Ror2 signaling [108].

CaSR negatively regulates cell proliferation by inhibiting β-catenin. Both global and intestine-specific CaSR KO mice have been shown to exhibit the hyperproliferative phenotypes of colonic epithelia concurrent with activation of Wnt/β-catenin signaling [109]. Importantly, calcimimetic R-568, a CaSR activator, has been demonstrated to effectively suppress β-catenin transcriptional activity by reducing phosphorylation of β-catenin at Ser^{522} and Ser^{675} [109]. Furthermore, the role of CaSR in inducing apoptosis in IEC has previously been documented. Treatment with R-568 increased an apoptotic rate by enhancing caspase 3/7 activity in HT-29 and Caco-2 CRC cell lines [110]. Interestingly, this proapoptotic effect of R-568 was absent in the IEC expressing a dominant negative CaSR mutant [110,111]. All of these findings indicate that CaSR plays important roles in regulating the differentiation, proliferation and apoptosis of IEC.

Regulation of intestinal ion transport by CaSR

CaSR has been proposed as a modulator of intestinal ion transport. Using the pH stat technique and short-circuit current measurement, it has been demonstrated

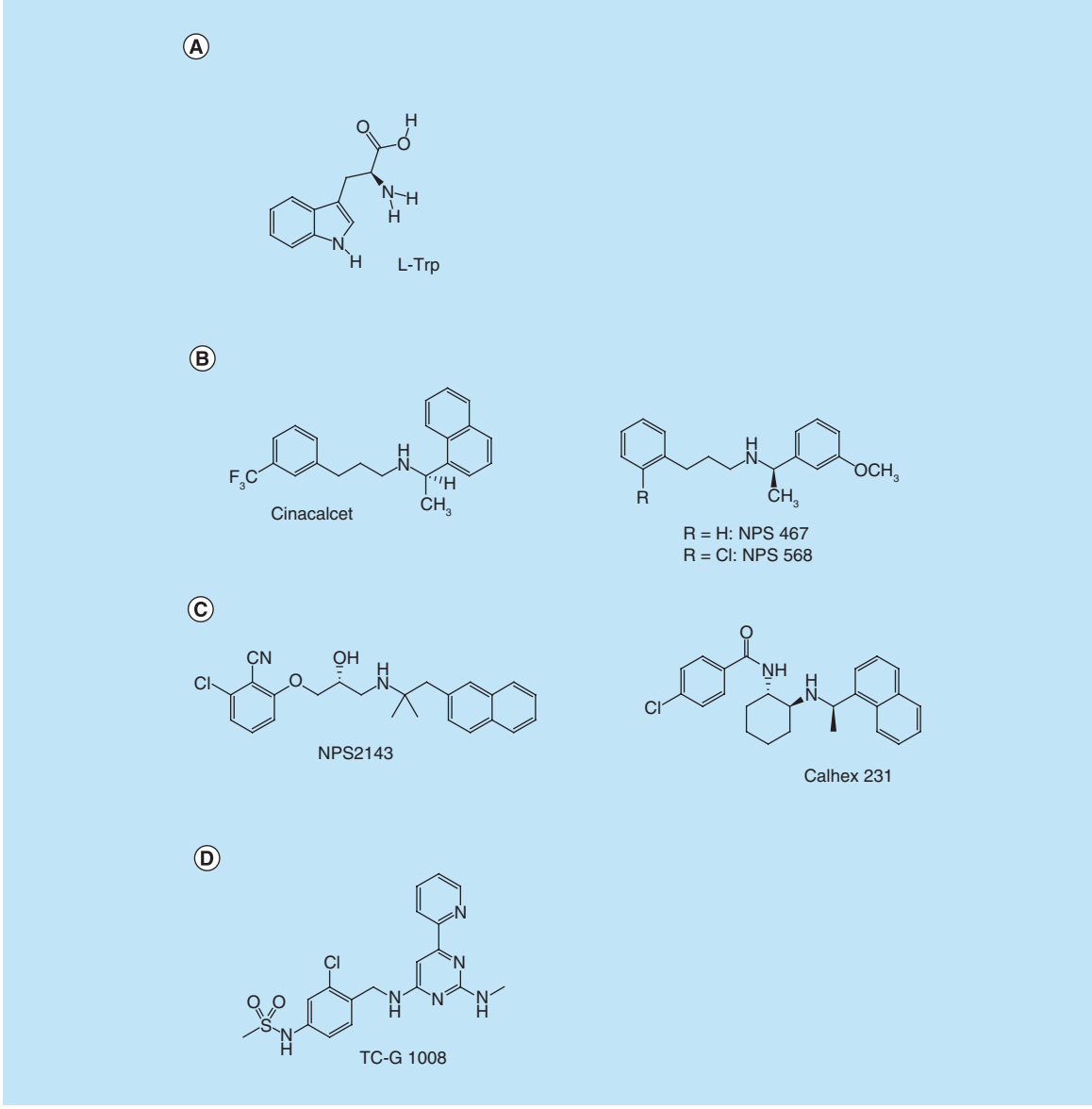

Figure 7. Chemical structures of ligands of calcium-sensing receptors and GPR39. (A) CaSR agonists. **(B)** CaSR antagonists. **(C)** GPR39 agonists. CaSR: Calcium-sensing receptor.

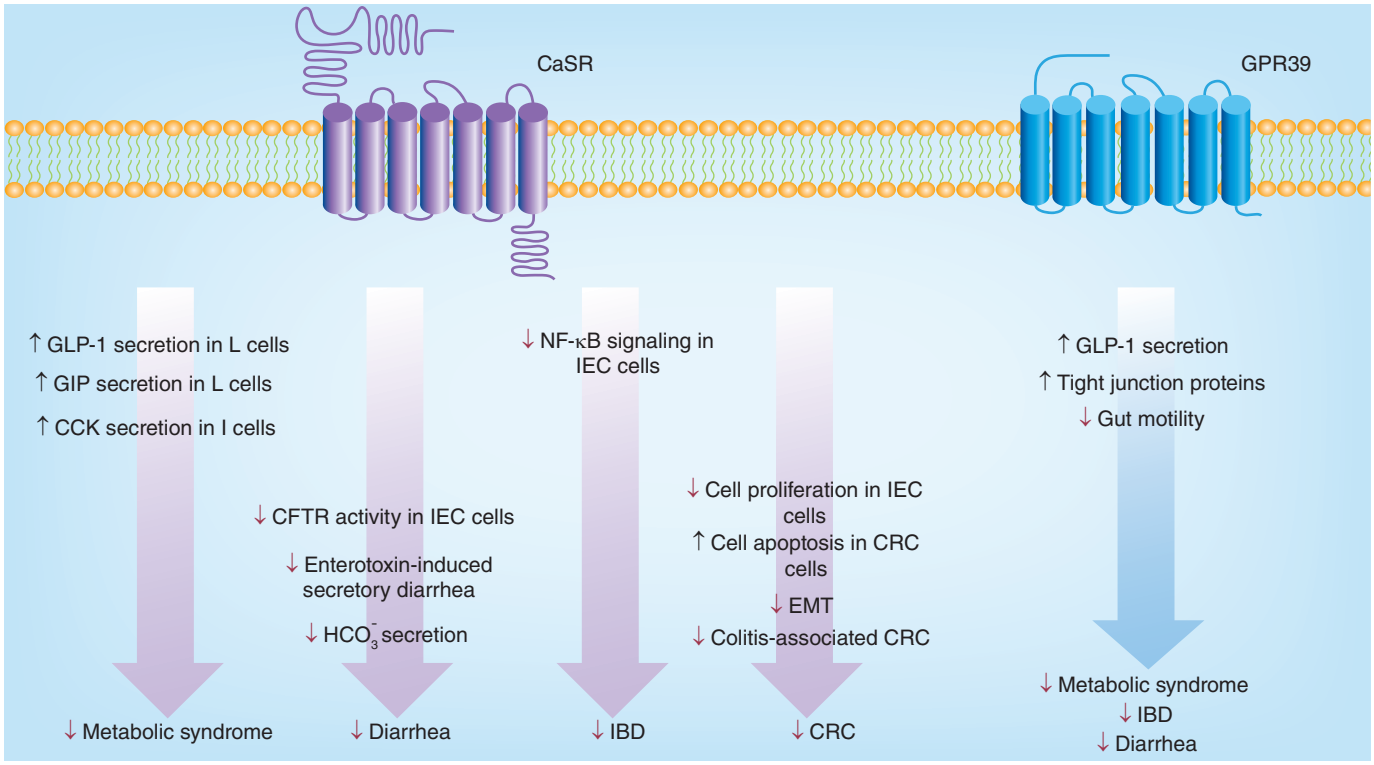

Figure 8. Physiological and pathological roles of mineral receptors in GI health and disease. Stimulation of CaSR leads to gut hormone secretion, inhibition of CFTR-mediated intestinal fluid secretion and suppression of intestinal inflammation and cell proliferation. It has been proposed as a drug target for metabolic syndrome, IBD, CRC and diarrhea. In addition, GPR39 mediates the signal to activate GLP-1 secretion and promote intestinal tight junction integrity as well as inhibit GI motility. Therefore, based on the physiological roles of GPR39, GPR39 is regarded as a drug target for T2DM, IBD and inflammation-associated diarrhea.
CaSR: Calcium-sensing receptor; CFTR: Cystic fibrosis transmembrane conductance regulator; CRC: Colorectal cancer; GLP-1: Glucagon-like peptide-1; IBD: Inflammatory bowel disease; IEC: Intestinal epithelial cells.

that a CaSR agonist R-568 stimulated both Cl^-- and SCFA-dependent HCO_3^- secretion [112]. In mice, the mechanism of CaSR-induced duodenal HCO_3^- secretion is via a receptor-operated, channel-mediated elevation in intracellular Ca^{2+} and the subsequent opening of Ca^{2+}-activated K^+ channels [113].

The cystic fibrosis transmembrane conductance regulator (CFTR) is a cAMP-activated Cl^- channel involved in intestinal Cl^- secretion [114]. CFTR activity can be inhibited by phosphodiesterase (PDE)-mediated cAMP hydrolysis and phosphorylation at its inhibitory site by AMPK [115,116]. CaSR has been found to be a negative regulator of CFTR channel activity [117]. This effect results from an increase in intracellular Ca^{2+} induced by CaSR stimulation, which subsequently suppresses CFTR activity via mechanisms involving stimulation of PDE [118] and AMPK activation [50].

Regulation of enteroendocrine function by CaSR

In addition to gastrin, CaSR stimulation modulates the secretion of other gut hormones. For instance, CaSR stimulation has been shown to induce GLP-1 and CCK secretion by mechanisms involving the $G\alpha_q$-PLC-induced calcium flux from IP_3 receptors, L-type Ca^{2+} channels and transient receptor potential channels [119]. In addition, R-568 induced GIP secretion in rat small intestine [120]. In agreement with this finding, the release of GIP upon the treatment with L-amino acids, which are CaSR agonists, was blocked by the CaSR inhibitor calhex 231 [120]. Therefore, CaSR is recognized as a regulator of gut hormone secretion.

Roles of CaSR as a drug target

Roles of CaSR in the modulation of intestinal inflammation

The intestinal epithelium-specific CaSR KO mice exhibited an IBD-like phenotype, including impaired intestinal barrier integrity and upregulation of inflammatory receptors, such as toll-like receptors and nod-like receptors (NOD) in intestinal epithelia [121]. Expression level of CaSR in the jejunum and ileum in the LPS-challenged piglet model was inversely correlated with expression of phospho-NF-κB p65, IKKα/β and IκB [122]. Additionally, activation of CaSR suppressed NF-κB signaling and expression of proinflam-

matory cytokines, including TNF-α, IL-1β, IL-6 and IL-8, and increased expression of anti-inflammatory cytokines, including IL-10 in mouse intestine [123]. Interestingly, the CaSR antagonist NPS-2143 abolished the anti-inflammatory effect of CaSR agonism in Caco-2 and HT-29 cells [101]. Intestinal epithelium-specific CaSR KO mice were more susceptible to DSS-induced colitis than the WT mice [121]. Importantly, stimulation of CaSR significantly alleviated the clinical signs of colitis, including weight loss, colon shortening and mucosal damage, in a mouse colitis model [123]. Taken together, these findings suggest that CaSR represents a drug target for the treatment of intestinal inflammatory conditions, especially IBD.

CaSR as a drug target for CRC

Intestinal inflammation is a risk factor for CRC [124]. Due to the anti-inflammatory effect of CaSR stimulation [121], it is hypothesized that CaSR agonists may be beneficial in the prevention of CRC. Interestingly, an inverse correlation between CaSR and the incidence of CRC has been reported [125]. Furthermore, the restoration of CaSR in both *in vitro* and *in vivo* models of CRC suppressed the malignant phenotypes of CRC via mechanisms involving inhibition of cell proliferation and an enhancement of the apoptosis of cancer cells [50,125]. In addition, it has been demonstrated that a marker of EMT was markedly upregulated concurrent with the malfunction and low expression levels of CaSR in intestinal-specific CaSR knockout mice [110]. These phenomena could also be observed in CRC patients [110]. Surprisingly, stable transfection with CaSR has been shown to suppress the proliferation of Caco-2 and HT-29 CRC cell lines [110]. In addition, CaSR agonism by chitosan oligosaccharide led to activation of AMPK in IEC and protected against colorectal tumorigenesis in a mouse model of colitis-associated CRC [50]. Therefore, CaSR has been considered as a tumor suppressor and recognized as a promising target for the prevention and treatment of CRC.

Targeting CaSR for the treatment of metabolic syndrome

Gut hormones, including GLP-1, CCK and PYY, are known to regulate the development of obesity [126]. Previous studies have reported the role of CaSR in regulating the secretion of these hormones [127]. Impaired incretin secretion is considered one of the important hallmarks of obesity and T2DM [30]. CaSR stimulation has been shown to increase the secretion of gut-derived hormones involved in metabolic regulation, especially GLP-1 [119]. Because GLP-1 is known to exert a therapeutic impact in T2DM and metabolic syndrome patients, it is envisioned that CaSR agonists may be beneficial in the treatment of metabolic syndrome. Future investigations are required to prove this hypothesis.

Targeting CaSR for antidiarrheal therapy

CFTR-mediated chloride secretion plays a key role in driving intestinal fluid secretion in secretory diarrhea, including cholera [128]. Activation of CaSR is known to reduce CFTR activity via a mechanism involving PDE-mediated cAMP degradation [129]. Therefore, stimulation of CaSR might be an effective strategy to reduce intestinal fluid loss in secretory diarrhea. Indeed, CaSR activation by specific agonists [118] and natural compounds [50] has been shown to ameliorate the severity of diarrhea in a mouse model of enterotoxin-induced secretory diarrheas [50]. In addition, stimulation of CaSR could also led to inhibition of intestinal HCO_3^- secretion, a process that is upregulated during secretory diarrhea [112]. Therefore, CaSR has been considered as a drug target for the treatment of secretory diarrhea. Currently, the CaSR agonist R-586 is in the preclinical phase of the development of antidiarrheal therapy [117].

Zinc-sensing receptor (GPR39)

Background on GPR39

GPR39 was first discovered in 1997. Later, it was found that Zn^{2+} specifically binds to GPR39 with high affinity activating intracellular signaling. Thereafter, GPR39 has been recognized as a Zn^{2+}-sensing receptor [3,4]. Indeed, human GPR39 gene is located on chromosome 2, position q21.2 [130]. There are two variants of the human GPR39 gene including GPR39-1a and GPR39-1b, which are considered to be active and inactive forms, respectively [131]. GPR39 is ubiquitously expressed in the body [130]. After being agonized by Zn^{2+}, GPR39 emanates its intracellular downstream signaling through $G\alpha_q$-PLCβ-IP3-Ca^{2+}, MAPK and PI3K pathways. GPR39 is considered an important antidepressant target. In addition, GPR39 agonism is known to produce several beneficial impacts on neuronal functions [132], skin wound healing [133], sperm capacitation [134] and myogenesis [135]. GPR39 is highly expressed in the apical membrane along the GI tract, especially in the colon [136] and plays significant roles in modulating GI functions and in the prevention/treatment of GI disorders (Figure 8). Recently, a 2-pyridylpyrimidine derivative TC-G 1008 has been discovered to be a potent GPR39 agonist (Figure 7). This compound potently agonizes both human and rat GPR39 with EC_{50} of <1 nM [137]. The biaryl moiety of the central pyrimidine ring of this compound is required for GPR39-stimulating activity [137]. Of note, TC-G 1008 do not compete for binding at the Zn^{2+}-binding sites, in other words, His-17 and His-19 in the extracellular domain [137,138].

Physiological roles of GPR39 & its role as a drug target

Regulation of gastric function & GI motility by GPR39

Using a pylorus ligation model, the volume of gastric juice has been found to increase without a change in gastric pH in GPR39 KO mice [139]. This result suggests that GPR39 promotes gastric secretion without affecting acid secretion. Because motilin and ghrelin receptors, which are known to stimulate GI motility, share similarity in their structure with GPR39 [130], it was hypothesized that GPR39 may play a role in the regulation of GI motility. Using the ^{14}C octanoic breath test, which monitors gastric emptying by measuring the production of $^{14}CO_2$, gastric emptying has been found to be significantly accelerated in GPR39 KO mice compared with wild-type mice [139]. In addition, using an *in vitro* model of smooth muscle stripped from sheep duodenum, Zn^{2+} inhibited both the frequency and amplitude of smooth muscle contraction in a dose-dependent fashion [140]. It is therefore believed that GPR39 stimulation inhibits GI motility. In addition, GPR39 agonists may be beneficial in the treatment of diseases associated with GI hypermotility, for example, irritable bowel syndrome. Likewise, GPR39 antagonists may be beneficial in the treatment of chronic constipation, dyspepsia, hypomotility-associated diarrhea and gastroparesis [130]. However, GPR39 antagonists have not been available to date.

GPR39 in the regulation of tight junction formation

Using transepithelial electrical resistance measurement to evaluate tight junction integrity, GPR39 silencing decreases transepithelial electrical resistance in Caco-2 cells along with a diminished expression of tight junction proteins, including ZO-1 and occludins [141]. Consistent with this finding, GPR39 KO mice exhibited abnormal membrane localization of tight junction proteins [141,142]. The mechanisms of GPR39-induced enhancement of tight junction integrity involve cell proliferation signaling pathways, including ERK- and AKT-activated mTOR/p70S6K signaling [142,143]. Because impaired intestinal barrier function is an important hallmark of IBD [24], GPR39 has been hypothesized to be a drug target of IBD. Indeed, GPR39 KO mice were more susceptible to DSS-induced colitis than WT mice [142]. Moreover, intestinal inflammation has been demonstrated to be associated with Zn^{2+} deficiency [144], and Zn^{2+} supplementation has been found to suppress the severity of diarrhea and intestinal inflammation [145]. Therefore, GPR39 agonists may find their therapeutic applications in the treatment of GI diseases associated with impaired tight junction integrity, including IBD and other inflammatory diarrheal diseases.

GPR39 & enteroendocrine functions

GPR39 KO mice have been found to exhibit impaired glucose tolerance and decreased plasma insulin in response to oral and intravenous administration of glucose [146]. Furthermore, GPR39 stimulation has been reported to increase insulin secretion by increasing insulin receptor substrate-2 expression [146]. However, GPR39 stimulation did not produce a direct effect on pancreatic insulin secretion *ex vivo* [146]. This indicates that GPR39 activation indirectly induces insulin secretion. In addition, the roles of GPR39 in the regulation of GLP-1 secretion have been demonstrated [137]. TC-G 1008 (Figure 7) has been proposed as the first orally available GPR39 agonist that stimulated the secretion of GLP-1 in mice (30 mg/kg P.O.) [137], which is known to induce pancreatic β-cell proliferation and potentiate insulin secretion [30]. Therefore, GPR39 represents a promising drug target for the treatment of diabetes patients.

Conclusion

Fatty acid and mineral receptors play key roles in regulating several physiological processes in the GI tract, including gastric secretion, GI motility, secretion of gut hormones, cell proliferation and apoptosis and ion transport. These receptors belong to the GPCR superfamily. They represent promising drug targets for several GI disorders including inflammatory bowel disease, colorectal cancer and diarrheas. In addition, these receptors regulate secretion of gut hormones especially GLP-1 and, therefore, serve as drug targets for metabolic syndrome.

Fatty acid and mineral receptors have been found to modulate GI function and are regarded as promising therapeutic targets for CRC, metabolic syndrome, IBD and diarrhea. Stimulation of CaSR and FFA2 – FFA4 can decrease cell proliferation in both *in vitro* and *in vivo* models of CRC. In addition, the agonism of CaSR, GPR39 and LCFA receptors induces GLP-1 secretion, which is of therapeutic benefit in the treatment of metabolic syndrome. However, *in vivo* efficacy testing in suitable animal/human models is required to prove that the agonists of these receptors can be effective in the treatment of CRC and metabolic syndrome. Furthermore, these fatty acid and mineral receptors represent promising therapeutic targets of IBD. Indeed, GLPG0974, a synthetic ligand of FFA2, has been entered into Phase I clinical trials for IBD treatment. Because GPR39 agonists have been shown to reduce the magnitude of intestinal motility, further studies should be performed to evaluate their potential utility in the treatment of IBS in animal models or human models. GPR39 antagonists may also be useful for treating constipation. However, GPR39 antagonists are not available at present. Future effort should be made to identify novel GPR39 antagonists

and evaluate their potential applications in the treatment of constipation. In summary, further investigations should be directed to provide a proof-of-principle for the therapeutic benefits of these nutrient-sensing receptors in the treatment of GI disorders using animal models or human models. Specific agonists and antagonists of these receptors will be required to attain this goal.

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Executive summary

- Fatty acid and mineral receptors are proposed as drug targets for GI disorders and metabolic syndrome, whose pathogenesis involves altered gut microbiota.Inflammatory bowel disease
- Accumulated lines of evidence have shown that targeting these receptors reduced the severity of colitis. In experimental models of colitis, the FFA4 ligand TUG-891 inhibited NF-κB-mediated intestinal inflammation, whereas calcium-sensing receptors (CaSR) KO mice and GPR39 KO mice were more susceptible to colitis, suggesting that activation of these receptors may be useful in the treatment of inflammatory bowel disease. In contrast, stimulation of FFA2 and FFA3 induced inflammatory responses, and inhibition of these short-chain fatty acid (SCFA) receptors attenuated the severity of colitis. Indeed, the FFA2 antagonist GLPG0974 reduced intestinal inflammation in colitis models and has undergone Phase I clinical trials for the treatment of inflammatory bowel disease.

Colorectal cancer

- Stimulation of SCFA receptors, including FFA2 and FFA3, led to reduced expression of cell cycle regulators, including cyclin D3 and cyclin-dependent kinase, and increased expression of p21. In addition, stimulation of SCFA receptors reduced inflammatory signaling, which is known to promote hyperproliferative phenotypes of colorectal cancer. Activation of LCFA receptor FFA4 and CaSR also suppressed EMT markers. Therefore, FFA4 and CaSR have been considered as therapeutic targets of colorectal cancer.

Metabolic syndrome

- Activation of fatty acid receptors, including FFA1–FFA4, induces secretion of gut hormones, including GLP-1, which is known to be a drug target for obesity and T2DM. Similarly, stimulation of CaSR has been reported to trigger GLP-1 secretion and other appetite-suppressing hormones, including CCK and PYY. Activation of the Zn^{2+}-sensing receptor GPR39 by 2-pyridylpyrimidines also stimulated GLP-1 secretion resulting in pancreatic β-cell proliferation in mice. Altogether, activation of these receptors represents a potential therapeutic approach for the treatment of metabolic syndrome.

Diarrhea

- Activation of CaSR by a synthetic agonist R-586 has been shown to inhibit CFTR-mediated intestinal Cl^- secretion, which is involved in the pathogenesis of diarrhea via mechanisms involving activation of PDE. Moreover, zinc supplementation is known to reduce the severity of diarrheal diseases. It has been hypothesized that GPR39 mediates the antidiarrheal effect of zinc at least in part by enhancing intestinal barrier integrity and inhibiting intestinal motility. Therefore, agonists of CaSR and GPR39 may find their application in the treatment of diarrhea.

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