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Pentacyclic triterpenes modulate farnesoid X receptor expression in colonic epithelial cells: Implications for colonic secretory function

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The nuclear bile acid receptor, farnesoid X receptor (FXR), is an important regulator of intestinal and metabolic function. Previous studies suggest that pentacyclic triterpenes (PCTs), a class of plant-derived bioactive phytochemical, can modulate FXR activity and may therefore offer therapeutic benefits. Here, we investigated the effects of a prototypical PCT, hederagenin (HG), on FXR expression, activity, and antisecretory actions in colonic epithelial cells. T_{84} cells and murine enteroid-derived monolayers were employed to assess HG effects on FXR expression and activity in colonic epithelia. We measured mRNA levels by qRT-PCR and protein by ELISA and immunoblotting. Transepithelial Cl^- secretion was assessed as changes in short circuit current in Ussing chambers. We determined HG treatment (5–10 μM) alone did not induce FXR activation but significantly increased expression of the receptor, both in T_{84} cells and murine enteroid-derived monolayers. This effect was accompanied by enhanced FXR activity, as assessed by FGF-15/19 induction in response to the synthetic, GW4064, or natural FXR agonist, chenodeoxycholic acid. Effects of HG on FXR expression and activity were mimicked by another PCT, oleanolic acid. Furthermore, we found FXR-induced downregulation of cystic fibrosis transmembrane conductance regulator Cl^- channels and inhibition of transepithelial Cl^- secretion were enhanced in HG-treated cells. These data demonstrate that dietary PCTs have the capacity to modulate FXR expression, activity, and antisecretory actions in colonic epithelial cells. Based on these data, we propose that plants rich in PCTs, or extracts thereof, have excellent potential for development as a new class of "FXR-targeted nutraceuticals".

Bile acids (BAs) are molecules that are synthesized in the liver from cholesterol and stored, conjugated to either glycine or taurine, in the gallbladder. Upon eating a meal, they are released into the proximal small intestine, where they perform their classical functions in facilitating the digestion and absorption of dietary lipids. When they reach the terminal ileum, conjugated BAs are reabsorbed via the apical sodium-dependent bile salt transport and recycled back to the liver. However, with each cycle of this enterohepatic circulation (EHC), a small amount (~5%) of the circulating BA pool enters the colon and undergoes metabolism by the resident microbiota. The first step in the bacterial metabolism of BAs is hydrolysis of the taurine or glycine moiety to yield the unconjugated BA that is more lipophilic and membrane permeable than its conjugated counterpart. This is a vitally important process since, unlike the terminal ileum, there are no apical transporters for BAs in the colon, and their only way to enter the epithelium is by passive diffusion across the apical membrane. When BAs enter the colonic epithelium, they then have the capacity to activate the nuclear BA receptor, farnesoid X receptor (FXR).

FXR was first recognized as a receptor for BA in 1999 (1, 2) and along with this discovery came a growing appreciation that BAs are not only important for facilitating lipid digestion but that they are in fact a family of steroid hormones that regulate many aspects of human physiology (3–5). The most potent of the endogenous ligands of FXR is chenodeoxycholic acid (CDCA), followed by deoxycholic acid and lithocholic acid. When activated in the cytosol, FXR dimerizes with the retinoid X receptor and is translocated to the nucleus where it binds to FXR response elements to regulate gene transcription. In the small intestine, activation of epithelial FXR induces (or represses) the transcription of several genes, including FGF-19, that play critical roles in mediating enterohepatic feedback regulation of BA synthesis and transport (6, 7).

FXR is expressed in the colonic epithelium to a lesser extent than in the small intestine and although it is not known if it contributes to regulation of the EHC, it has been shown to have other important roles in regulating colonic epithelial function. Several studies have shown that FXR activation promotes colonic epithelial barrier function (8–14), an effect

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that likely contributes to its actions in preventing intestinal inflammation in various murine models of disease (14–16). Such findings suggest that FXR agonists may be of use in treating patients with inflammatory bowel disease. In addition to its effects in promoting barrier function, previous studies from our lab have shown FXR to be an important regulator of epithelial transport function (17). We found that activation of FXR prevents luminal fluid accumulation and the onset of diarrhea in mouse models of disease, effects that are due, at least in part, to downregulated expression of the cystic fibrosis transmembrane conductance regulator (CFTR) Cl^- channel in the apical membrane. Since Cl^- secretion through CFTR is the predominant driving force for fluid secretion in the colon, such actions of FXR make it an excellent target for the development of new drugs to treat secretory diarrheas, such as those brought about by enteric pathogens, allergens, and the malabsorption of BAs.

Although identified as a receptor for BAs more than 20 years ago, development of selective agonists for FXR has been slow, with just one semisynthetic ligand of the receptor, obeticholic acid (6-ethyl-CDCA), having been approved for clinical use to treat primary biliary cholangitis (18). No FXR-targeted drugs have yet been approved to treat intestinal disease, although clinical trials have shown obeticholic acid to be effective in alleviating symptoms of BA diarrhea (19). An alternative approach to targeting FXR for therapeutic purposes could come through the use of naturally occurring plant phytochemicals. In particular, pentacyclic triterpenes (PCTs), a class of phytochemical found in many plant species, have previously been shown to modulate FXR activity in hepatocytes (20–22). This raises the possibility that plant extracts rich in PCTs could have the capacity to be developed as therapeutic modulators of FXR activity. However, whether PCTs have the capacity to regulate FXR activity in the colonic epithelium is currently unknown. Thus, in the current study, we set out to address this gap in our knowledge by investigating the effects of a prototypical PCT, hederagenin (HG), on FXR activity and expression in colonic epithelial cells and possible implications for epithelial secretory function.

Results

HG is not an agonist of FXR in colonic epithelial cells

To determine the effects of PCTs on FXR signaling in colonic epithelial cells, we first investigated the actions of the prototypical PCT, HG (Fig. 1), on FXR activity. T_{84} cell monolayers were serum-starved for 24 h prior to treatment bilaterally with either DMSO (1%), GW4064 (5 μM), or HG (1–100 μM) for 24 h. While the positive control, GW4064, significantly upregulated both FGF-19 protein and mRNA expression, HG had no effect (Fig. 2, *A* and *B*). Low concentrations of HG (5–10 μM) had no significant effect on transepithelial electrical resistance (TEER), but high concentrations (50–100 μM) significantly decreased TEER by 72.7 ± 14.3 and 82.4 ± 36.8%, respectively, in comparison to a 7.9 ± 8.6% decrease in untreated control cells (n = 7; $p < 0.001$) (Fig. 2*C*). Such reductions in TEER suggest HG may exert toxic effects at high concentrations and in subsequent experiments, we therefore employed lower concentrations of the PCT that did not alter TEER. Further investigations of the effects of HG on FXR activation were conducted in FXR-luciferase reporter cells. Hep2G cells, stably expressing an FXR-luciferase reporter vector, were treated with either GW4064 (5 μM) or HG (1–10 μM) for 24 h. Luminescence was then measured as a marker of FXR activation. Similar to their actions in T_{84} cells, GW4064 robustly stimulated FXR activity, whereas treatment with HG was without effect (Fig. 2*D*).

HG upregulates FXR expression in colonic epithelial cells

Although it was without effect on FXR activity, we next investigated the effects of HG on expression of the receptor. T_{84} cells were serum-starved for 24 h prior to bilateral treatment with either DMSO (0.1%) or HG (5–10 μM) for 24 h. We found that HG (5–10 μM) significantly upregulated FXR mRNA expression compared to untreated controls (Fig. 3*A*). Analysis of the time course over which HG (5 μM) stimulates FXR mRNA expression revealed significant upregulation after 6 h with levels remaining elevated for 24 to 48 h (Fig. 3*B*). Analysis of FXR protein expression by Western blotting also demonstrated a time-dependent increase, with significant upregulation at 6 to 24 h (n = 7; $p < 0.05$) (Fig. 3*C*).

Having demonstrated HG to significantly upregulate FXR expression in T_{84} colonic epithelial cells, we next set out to examine if such effects were also apparent in *ex vivo* human colonic tissues. For these experiments, biopsies were taken from patients undergoing routine colonoscopy at Beaumont Hospital. Four normal biopsies were taken from the proximal colon of each patient. Biopsies were placed in a 24-well plate (2/well) containing media and were treated with either DMSO (0.05%) or HG (5 μM) for 6 h. FXR mRNA was analyzed by quantitative real-time PCR (qRT-PCR). Similar to our findings in cultured T_{84} cells, HG significantly upregulated FXR mRNA expression by 2.1 ± 0.9 fold over that of untreated control biopsies (n = 6; $p < 0.05$) (Fig. 3*D*).

PCTs such as HG can occur naturally in plants in both their glycosylated (saponin) and nonglycosylated (sapogenin) forms. Commonly occurring HG-related glycosides include α-hederin (α -H; Fig. 1) and hederacoside C (HC; Fig. 1) (23–25). Since glycosides are more hydrophilic than their nonglycosylated counterparts, they are predicted to have limited permeability across cell membranes, with the consequence that their bioactivity is likely to be altered. Thus, we investigated the effects of α-hederin and HC on FXR expression in colonic epithelial cells in comparison to HG. T_{84} cells were treated with either DMSO (0.05%), HG (5 μM), α-hederin (5 μM), or HC (5 μM) for 6 h. While HG significantly upregulated FXR mRNA expression (n = 4; $p < 0.05$), no significant change in expression of the receptor was observed in cells treated with either of the glycosides (Fig. 3*E*).

We also examined the effects of HG on another nuclear receptor known to be activated by BAs, the vitamin D receptor (VDR). However, in contrast to its effects on FXR, we found no

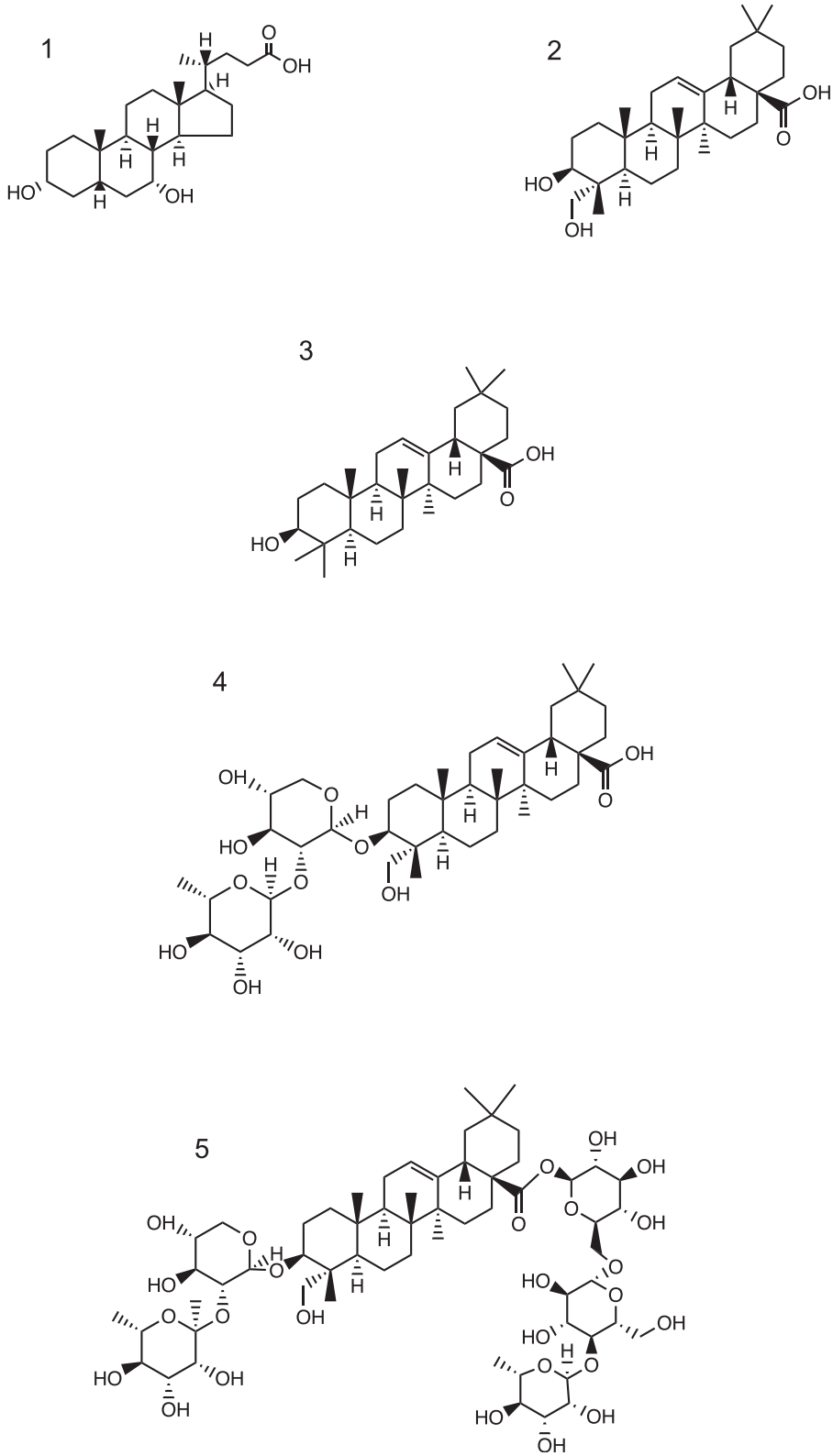

Figure 1. Chemical structures of the compounds of interest throughout this study. Carbon backbone and side chain structures of the bile acid chenodeoxycholic acid (1), the pentacyclic triterpenes hederagenin (2), oleanolic acid (3), the hederagenin-related glycosides, α-hederin (4), and hederacoside C (5).

significant change in VDR protein expression in cells treated with HG (Fig. 3*F*). HG was also apparently without effect on expression of the cell surface G protein–coupled bile acid receptor, TGR5, mRNA levels of which after treatment with HG (5 μM; 6 h) were found to be 1.3 ± 0.4 fold of those in control, untreated cells (n = 4; ns).

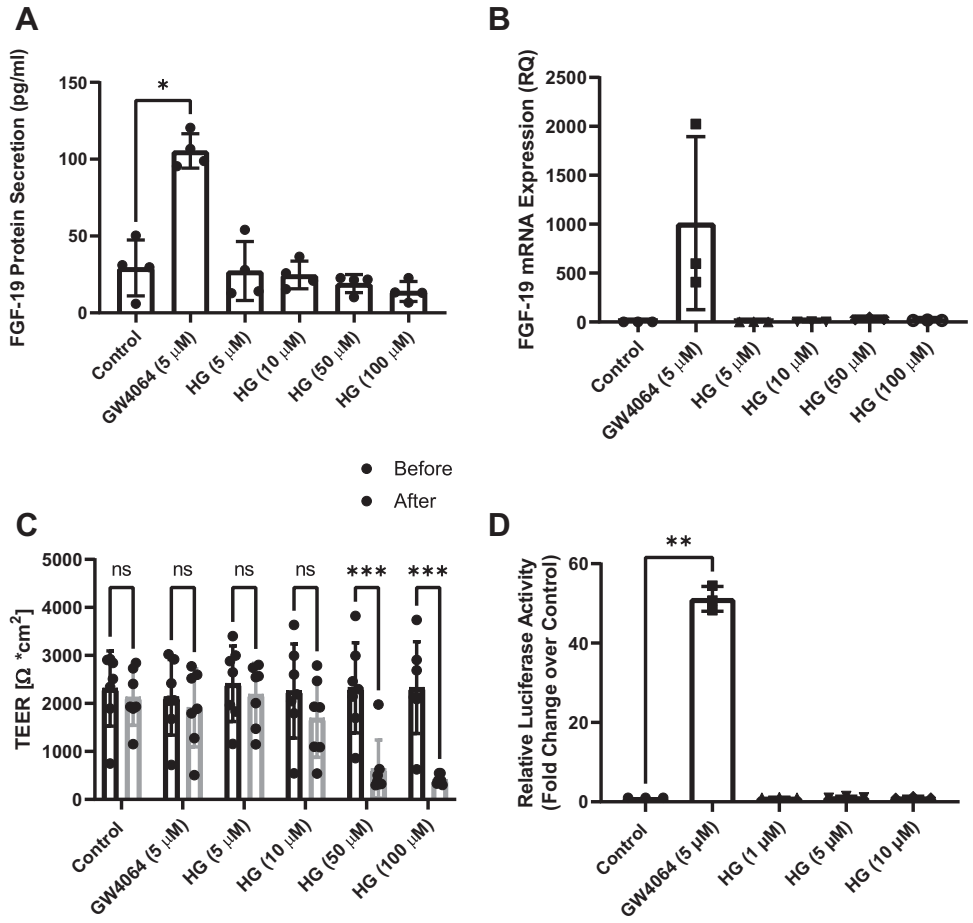

Figure 2. Hederagenin is not an agonist of FXR. T_{84} cells were serum-starved for 24 h and bilaterally treated with DMSO (1%), GW4064 (5 μM), or HG (5–100 μM) for 24 h. *A*, basolateral supernatants were collected, and FGF-19 protein was analyzed by ELISA (n = 4; *$p < 0.05$). *B*, FGF-19 mRNA expression was analyzed by qRT-PCR (n = 3). *C*, TEER was assessed pretreatment and posttreatment (n = 7; ***$p < 0.001$). *D*, FXR reporter cells were treated with DMSO (0.1%), GW4064 (5 μM), or HG (1–10 μM) for 24 h. FXR activation was assessed by luminescence measurement (n = 3; **$p < 0.01$). Data are expressed as mean ± SD. Statistical analysis was performed using either repeated measures one-way ANOVA with Dunnett's post hoc test or two-way ANOVA with Sidak's post hoc test, as appropriate. FXR, farnesoid X receptor; HG, hederagenin; qRT-PCR, quantitative real-time PCR.

HG potentiates agonist-induced FXR signaling in colonic epithelial cells

Since HG significantly upregulates colonic epithelial FXR expression, we next went on to investigate if treatment with the phytochemical alters epithelial responsiveness to agonists of the receptor. T_{84} cells were pretreated with HG (5 μM) for 1 h prior to treatment with GW4064 at a range of concentrations (0.5–5 μM) for 24 h. As expected, GW4064 induced both FGF-19 mRNA and protein expression in a concentration-dependent manner, whereas HG treatment alone was without effect. However, pretreatment of the cells with HG prior to GW4064 tended to enhance GW4064-induced FGF-19 mRNA expression, with statistically significant effects observed at the highest concentration of GW4064 tested (5 μM) (Fig. 4*A*). Effects of HG were even more apparent at the protein level where it significantly enhanced GW4064-induced FGF-19 secretion into the basolateral medium by 3.2 ± 1.9 ($p \leq 0.01$) and 2.6 ± 1.3 ($p \leq 0.001$) fold compared to responses observed in cells treated with 0.5 μM or 1 μM GW4064 alone (n = 9; Fig. 4*B*).

We also examined if the effects of HG on FXR signaling were apparent in the context of responses to a naturally occurring agonist of the receptor. For these experiments, we employed CDCA (Fig. 1), known to be the most potent of the endogenous BAs at FXR. We found that, similar to its effects on GW4064, pretreatment of T_{84} cells with HG significantly enhanced FGF-19 protein secretion into the basolateral medium in response to CDCA (50 μM; 24 h) (Fig. 4*C*).

Oleanolic acid enhances agonist-induced FXR signaling

To determine if enhancement of FXR signaling is a common feature of PCTs or if it is a specific effect of HG, we also investigated the effects of another PCT commonly found in plants, oleanolic acid (OA; Fig. 1). T_{84} cells, grown as monolayers on permeable supports, were serum-starved for 24 h prior to bilateral pretreatment with OA (5 μM) for 1 h. Cells were then bilaterally treated with GW4064 (5 μM) for 24 h. We found that similar to HG, while OA itself did not activate FXR, it significantly upregulated GW4064-induced FGF-19 protein secretion into the basolateral medium compared with cells treated with GW4064 alone (Fig. 4*D*).

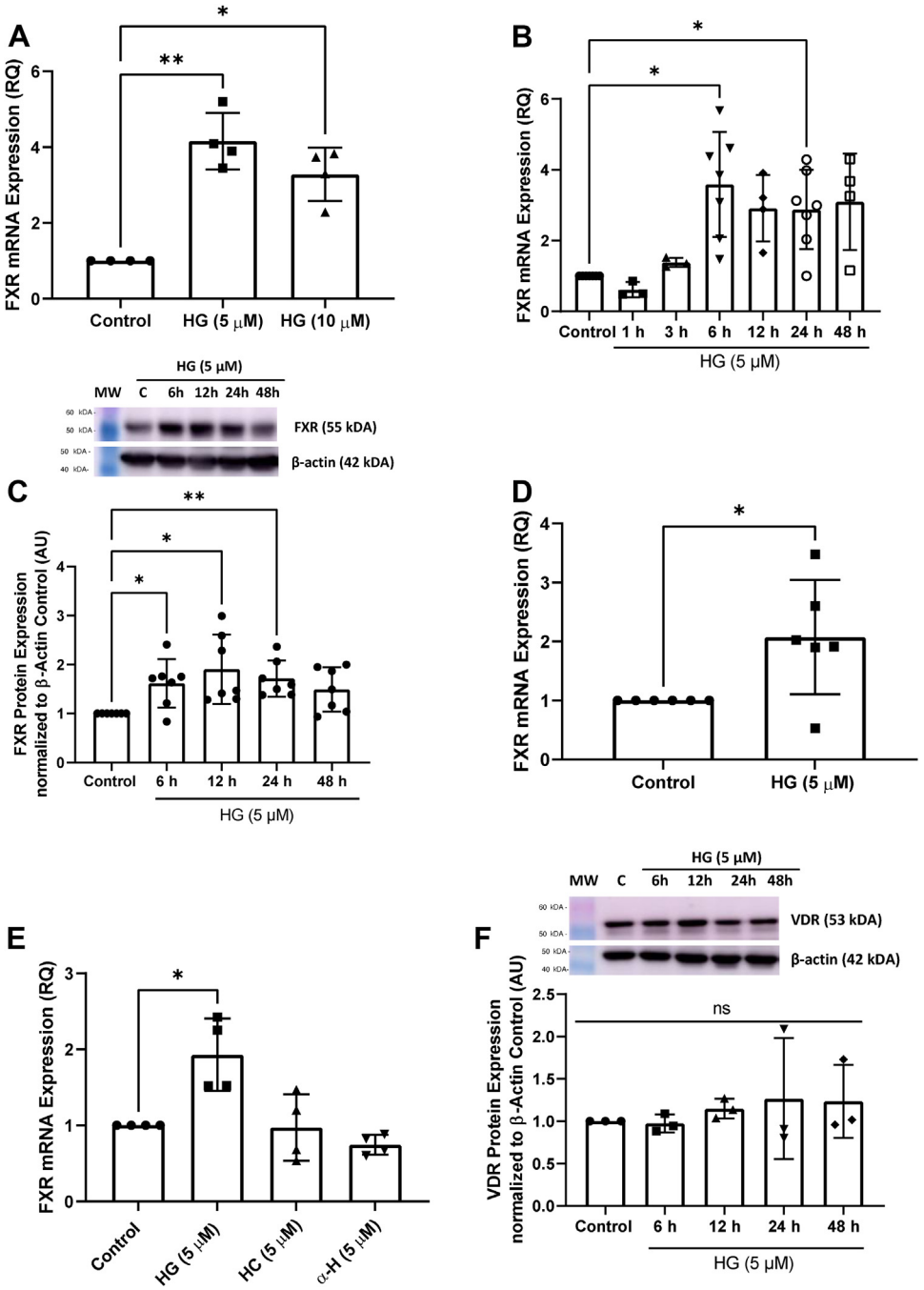

Figure 3. Hederagenin significantly upregulates FXR expression in colonic epithelial cells. T_{84} cells were serum-starved for 24 h and bilaterally treated with DMSO (0.05%–0.1%) or HG (5–10 μM) for 24 h. *A*, FXR mRNA expression was analyzed by qRT-PCR (n = 4; $^{**}p < 0.01$, $^{*}p < 0.05$). T_{84} cells were serum-starved for 24 h and bilaterally treated with DMSO (0.05%) or HG (5 μM) for a range of times. *B*, FXR mRNA expression was analyzed by qRT-PCR (n = 3–7; $^{*}p < 0.05$). *C*, representative Western blot of FXR protein expression with β-actin being used as the loading control. FXR protein expression was assessed by densitometry. All values were normalized to β-actin protein expression and expressed as fold change over vehicle-treated controls (n = 7; $^{*}p < 0.05$). *D*, human colonic biopsies were treated with DMSO (0.05%) or HG (5 μM) for 6 h. Tissues were collected and stored in RNA later. FXR mRNA expression was analyzed by qRT-PCR (n = 6; $^{*}p < 0.05$). *E*, T_{84} cells were serum-starved for 24 h prior to 6 h treatment with DMSO (0.05%), HG (5 μM), HC (5 μM), or α-H (5 μM). FXR mRNA expression was analyzed by qRT-PCR (n = 4; $^{*}p < 0.05$). *F*, T_{84} cells were serum-starved for 24 h and bilaterally treated with DMSO (0.05%) or HG (5 μM) for the times indicated. VDR protein expression was assessed by Western blotting. Representative Western blot image of VDR protein expression with β-actin being used as a loading control. VDR protein expression was assessed by densitometry. All values were normalized to β-actin protein expression and expressed as fold change over vehicle-treated controls (n = 3). Data are expressed as mean ± SD. Statistical analysis was performed using either the paired Student *t* test, the repeated measures one-way ANOVA with Dunnett's post hoc test, or mixed effect analysis with Dunnett's post hoc test, as appropriate. FXR, farnesoid X receptor; HG, hederagenin; VDR, vitamin D receptor; qRT-PCR, quantitative real-time PCR.

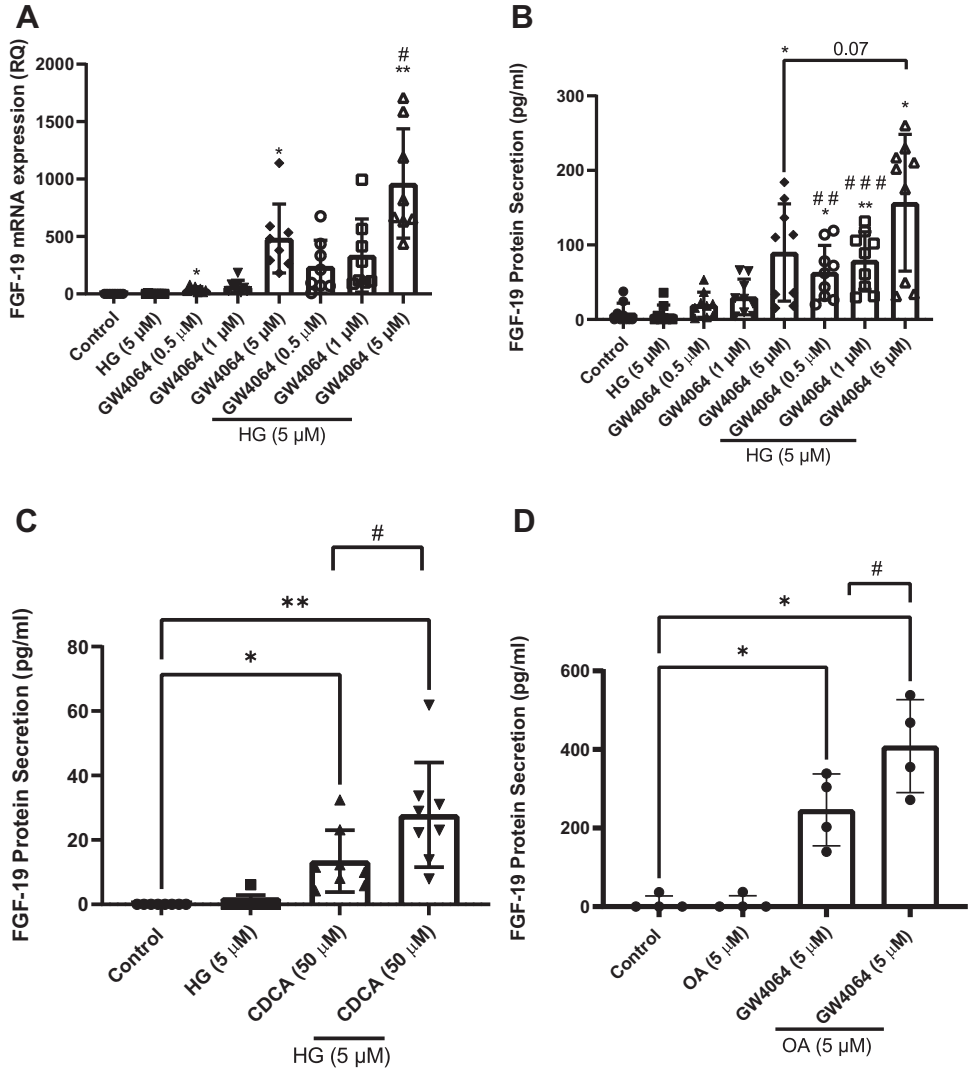

Figure 4. Pentacyclic triterpenes potentiate agonist-induced FXR signaling in T_{84} colonic epithelial cells. T_{84} cells were serum-starved for 24 h and bilaterally pretreated with HG (5 μM) for 1 h prior to 24 h treatment with varying concentrations of GW4064 (0.5–5 μM). *A*, FGF-19 mRNA expression was analyzed by qRT-PCR (n = 8; $^{**}p < 0.01$, $^{*}p < 0.05$, $^{\#}p < 0.05$). *B*, basolateral supernatants were collected and FGF-19 protein expression was assessed by ELISA (n = 9, $^{*}p < 0.05$). *C*, T_{84} cells were bilaterally pretreated with HG (5 μM) for 1 h prior to 24 h treatment with CDCA (50 μM). Basolateral supernatants were collected, and FGF-19 protein expression was assessed by ELISA (n = 8, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{\#}p < 0.05$). *D*, T_{84} cells were bilaterally pretreated with OA (5 μM) for 1 h prior to 24 h treatment with GW4064 (5 μM). Basolateral supernatants were collected, and FGF-19 protein expression was assessed by ELISA (n = 4, $^{*}p < 0.05$, $^{\#}p < 0.05$). Data are expressed as mean ± SD. Statistical analysis was performed using repeated measures one-way ANOVA with Tukey's post hoc test. *denotes significant differences compared to untreated controls, and $^{\#}$denotes significant differences compared to GW4064-treated cells. FXR, farnesoid X receptor; HG, hederagenin; OA, oleanolic acid; qRT-PCR, quantitative real-time PCR.

HG modulates FXR signaling in murine enteroid-derived monolayers

Data derived from T_{84} cell monolayers demonstrate that PCTs upregulate FXR expression and enhance agonist-induced FXR activation in colonic epithelial cells. Next, we sought to investigate if such effects are also apparent in more physiologically relevant primary cultures of colonic epithelial cells. In these experiments, murine enteroid-derived monolayers (EDMs) were pretreated with HG (5 μM: 1 h) prior to treatment with GW4064 (5 μM) for 24 h mRNA levels for FXR and FGF-15 (the murine ortholog to FGF-19) were then analyzed by qRT-PCR. Similar to our findings in T_{84} cells, HG significantly upregulated FXR mRNA expression (n = 4; $p < 0.01$) (Fig. 5*A*). Furthermore, HG pretreatment for 1 h prior to 24 h treatment with GW4064 significantly upregulated FGF-15 mRNA expression in comparison to GW4064 alone. Interestingly, in contrast to T_{84} cells, HG (5 μM) alone also significantly increased FGF-15 mRNA expression (n = 4; $p < 0.05$) (Fig. 5*B*).

HG enhances the antisecretory actions of FXR in colonic epithelial cells

Previous studies from our lab have shown that FXR activation exerts antisecretory effects on the colonic epithelium through downregulating expression of CFTR (17). Since our current data have shown PCTs to enhance FXR expression and signaling in colonic epithelia, we investigated if this might also have consequences for epithelial secretory function. Serum-starved

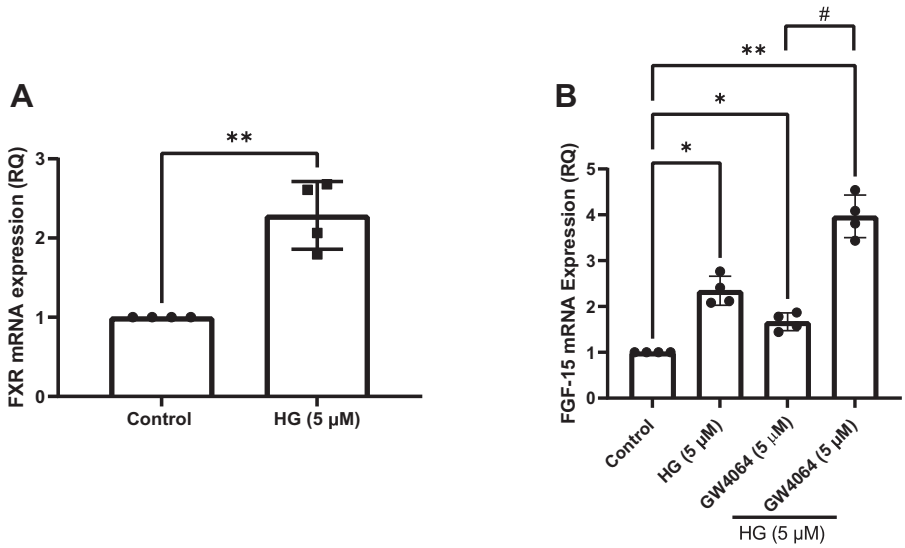

Figure 5. Hederagenin enhances FXR expression and signaling in murine colonic enteroids. Mouse colonic enteroids, grown as monolayers on permeable supports, were treated with HG (5 μM) for 1 h, prior to treatment with GW4064 (5 μM) for 24 h (*A*) FXR and (*B*) FGF-15 mRNA expression were analyzed by qRT-PCR (n = 4; *$p < 0.05$, **$p < 0.01$, $^{\#}p < 0.05$). Data are expressed as mean ± SD. Statistical analysis was performed using paired Student *t* test or repeated measures one-way ANOVA with Tukey's post hoc test. *denotes significant differences compared to untreated controls, and $^{\#}$denotes significant differences compared to GW4064-treated cells. FXR, farnesoid X receptor; HG, hederagenin; qRT-PCR, quantitative real-time PCR.

T_{84} cells were pretreated with HG (5 μM) for 1 h prior to treatment with GW4064 at a range of concentrations (0.5, 1, or 5 μM) for 24 h. While GW4064 (5 μM) significantly downregulated CFTR expression, lower concentrations of the agonist (0.5 and 1 μM) were without effect. However, in cells pretreated with HG, significant downregulation of CFTR mRNA was observed across all concentrations of HG tested (Fig. 6*A*). A similar trend was observed at the protein level. GW4064 (5 μM) significantly downregulated CFTR protein (n = 9; $p < 0.001$), whereas lower concentrations of the agonist (0.5–1 μM) were without effect. However, when cells were pretreated with HG prior to GW4064 (0.5 and 1 μM), CFTR was downregulated (n = 9). Furthermore, when compared to cells treated with GW4064 (0.5 and 1 μM) alone, HG enhanced downregulation of CFTR protein ($p < 0.01$ in each case, n = 9) (Fig. 6*B*).

In similar experiments, we investigated the effects of HG on CFTR mRNA expression in murine EDMs. EDMs were pretreated with HG (5 μM; 1 h) prior to 24 h treatment with GW4064 (5 μM). Similar to its effects on T_{84} cells, GW4064 alone significantly downregulated CFTR mRNA expression, while pretreatment with HG significantly enhanced the effect of GW4064. Treatment with HG alone did not alter CFTR mRNA expression compared to control cells (Fig. 6*C*).

Finally, based on our findings that HG enhances the effects of FXR activation on CFTR expression *in vitro*, we set out to determine if the PCT also modulates agonist-induced epithelial secretory responses. T_{84} cells were pretreated with HG (5 μM) for 1 h prior to treatment with GW4064 (5 μM) for 24 h. After 24 h of treatment, cell monolayers were mounted in Ussing chambers and Cl^- secretory responses to the cAMP-dependent agonist, forskolin (FSK) (10 μM), were measured as changes in short circuit current (I_{sc}). In keeping with previously reported findings (17), GW4064 (5 μM) significantly reduced FSK-induced I_{sc} responses to approximately 50% of those in untreated control cells. While treatment with HG alone was without effect, in cells pretreated with the PCT, the antisecretory actions of GW4064 were significantly enhanced (Fig. 6, *D* and *E*).

Discussion

Over the past 20 years, FXR has emerged as an important regulator of many aspects of our physiology. Originally identified as being a key regulator of BA synthesis and EHC, FXR is now also appreciated as being important in modulating many aspects of intestinal function. Reduced expression of epithelial FXR is associated with the development of colorectal cancer and inflammatory bowel disease, while its activation has been shown to be protective against increased epithelial permeability, intestinal inflammation, and tumor growth in mouse models of disease (5). FXR is also recognized as a promising target for the treatment of metabolic disorders (26), while our own studies have shown it to exert antidiarrhoeal actions (17). With the global incidence of intestinal and metabolic disease on the rise, it is clear that safe and effective new therapies are urgently needed. Here, we demonstrate that pentacyclic triterpenes, phytochemicals commonly found in dietary plants (27–29), have the potential to be developed as a new approach to modulate colonic epithelial FXR for disease treatment.

To begin investigating effects of PCTs on FXR signaling in colonic epithelial cells, we used the oleanane PCT, HG. Initially discovered in 1849 in English Ivy seeds, HG is reported to exert anticancer and anti-inflammatory actions (30–33) and is present in many plants that are commonly used in traditional medicines for conditions such as diabetes, obesity, and inflammatory disorders (24, 34–37). Given that PCTs have previously been demonstrated to activate FXR in other cell types (21, 22, 38), we first investigated if they may

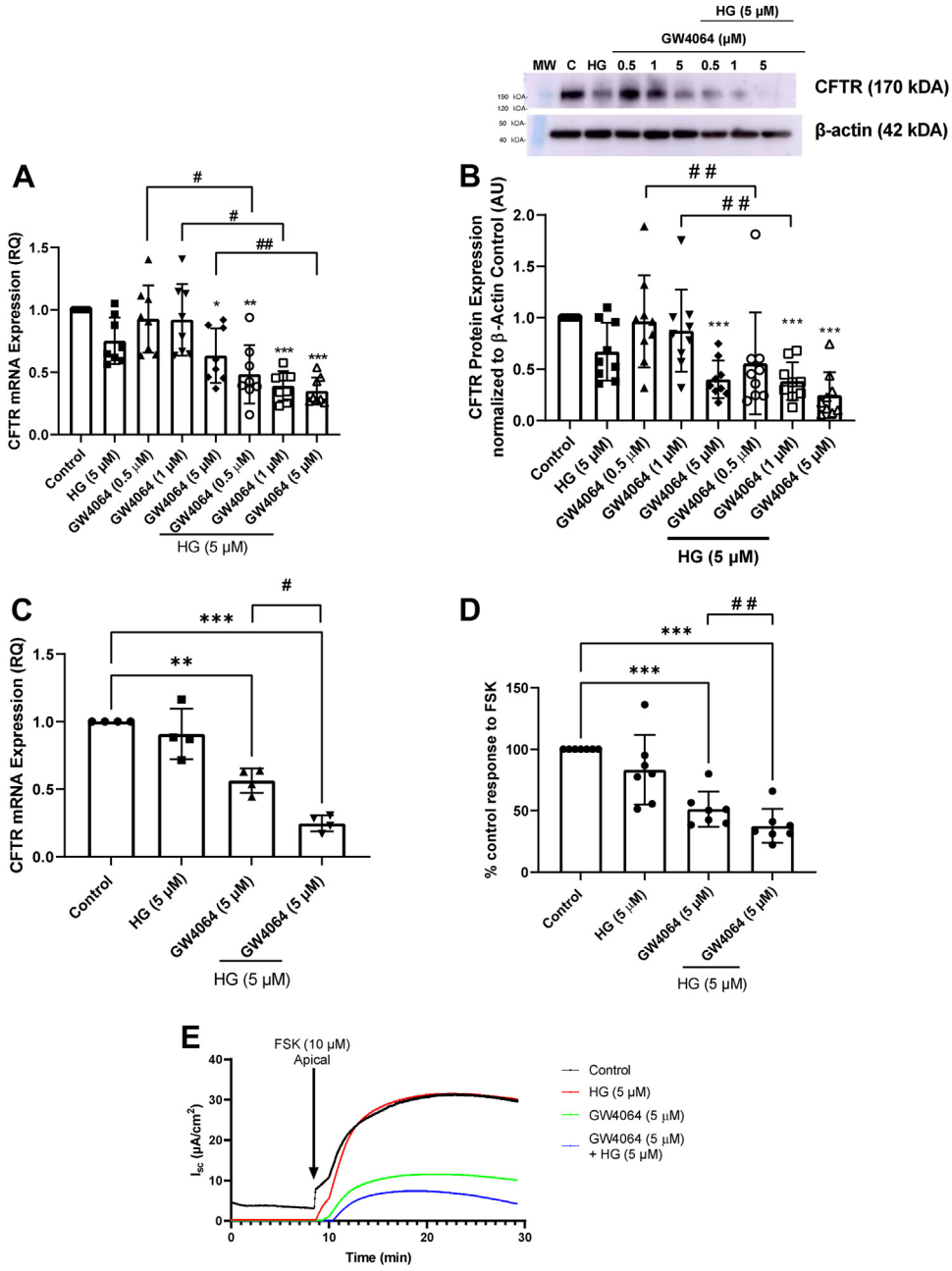

Figure 6. Hederagenin significantly enhances FXR-inhibition of CFTR expression and chloride secretion. T_{84} cells were serum-starved for 24 h and bilaterally pretreated with HG (5 μM) for 1 h prior to 24 h treatment with varying concentrations of GW4064 (0.5–5 μM). *A*, CFTR mRNA expression was analyzed by qRT-PCR (n = 8; $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#}p < 0.05$). *B*, representative Western blot of CFTR protein expression with β-actin being used as the loading control. CFTR protein expression was assessed by densitometry. All values were normalized to β-actin protein expression and expressed as fold change over vehicle-treated controls (n = 9; $^{***}p < 0.001$, $^{\#\#}p < 0.01$). *C*, murine colonic enteroids were pretreated with either HG (5 μM) or DMSO (0.05%) for 1 h, prior to 24 h treatment with GW4064 (5 μM). CFTR mRNA expression was analyzed by qRT-PCR (n = 4; $^{***}p < 0.001$, $^{**}p < 0.01$, $^{\#}p < 0.05$). *D*, T_{84} cells monolayers were bilaterally pretreated with HG (5 μM) for 1 h prior to 24 h treatment with GW4064 (5 μM). Cells were then mounted in Ussing chambers and voltage-clamped to zero potential difference for measurements of short circuit current (I_{sc}). Cells were apically treated with FSK (10 μM) and changes in I_{sc} were monitored using Acquire and Analyse software (n = 7; $^{***}p < 0.001$, $^{**}p < 0.01$, $^{\#\#}p < 0.01$). *E*, representative I_{sc} traces of the data shown in panel (*D*). Data are expressed as mean ± SD. Statistical analysis was performed using repeated measures one-way ANOVA with Tukey's post hoc test. *denotes significant differences compared to untreated controls, and $^{\#}$denotes significant differences compared to GW4064-treated cells. CFTR, cystic fibrosis transmembrane conductance regulator; FSK, forskolin; FXR, farnesoid X receptor; HG, hederagenin; qRT-PCR, quantitative real-time PCR.

have similar effects in colonic epithelial cells. However, using FGF-19 expression as an index of FXR activation, we found HG to be ineffective in comparison to robust induction of the gene by the synthetic FXR agonist, GW4064. Another common dietary PCT, OA, was also without effect, suggesting that unlike their actions in some other cell types, PCTs do not act as agonists of FXR in human colonic epithelial cells. To study FXR agonism in more detail, we used a commercially available FXR-luciferase reporter cell line. While responsive to both GW4064 and the natural FXR agonist, CDCA, HG was also without effect on FXR activation in this model. Thus, our data suggest that the presence of FXR alone is not sufficient for

agonistic activity to be apparent in response to PCTs and that other, as yet unidentified cofactors, appear to be required.

Rather than being direct agonists of the receptor, our current findings suggest that PCTs act to regulate signaling via colonic epithelial FXR through modulating expression of the receptor. Treatment of T_{84} cells with HG increased expression of FXR mRNA and protein in a concentration- and time-dependent fashion. Furthermore, this increased receptor expression was associated with enhanced cellular responsiveness to subsequent treatment with either GW4064, as a synthetic agonist, or CDCA, as a natural agonist of FXR. Such actions were not specific to HG but were also observed upon treatment of the cells with OA. We also observed enhanced FXR expression in human colonic biopsies and in murine EDMs upon stimulation with HG, suggesting PCTs have the capacity to upregulate intestinal FXR signaling across different models and species. Interestingly, we found that HG treatment alone tended to increase FXR activity, as measured by FGF-15 expression, in murine EDMs. The reasons for such a difference between human and murine colonic epithelial cells are not clear but could indicate that, in addition to modulating receptor expression, HG may also act directly as an FXR agonist in mice.

Given its important roles in regulating epithelial function, mucosal immunity, and metabolism, the potential for the regulation of intestinal epithelial FXR by dietary phytochemicals has clear physiological and pathophysiological implications. In the present studies, we began to investigate physiological implications of PCT-induced FXR expression by examining its effects on epithelial fluid and electrolyte transport. In the intestine, Cl^- secretion is the primary driving force for intestinal fluid secretion and we have previously shown that FXR activation inhibits this process by downregulating the expression of CFTR Cl^- channels in the apical membrane. Our present studies show that increased FXR expression in response to HG treatment was associated with enhanced inhibition of FSK-induced Cl^- secretory responses in Ussing chambers. Using a range of concentrations of GW4064, we found that HG enhanced the antisecretory actions of the agonist, an effect that was correlated with enhanced downregulation of CFTR expression. These data suggest that diets rich in PCTs may be associated with reduced fluid secretion into the stool and may be useful as a new therapeutic approach for patients with secretory diarrheas. Indeed, it is interesting to note that the PCTs, OA, ursolic acid, and betulinic acid have previously been identified as active components in Chaenomeles extract, a Chinese traditional medicine used to treat diarrhea (39).

However, when considering the potential therapeutic benefits of PCT-containing foods or nutraceuticals in enhancing colonic epithelial FXR expression, one must also consider their bioavailability. In this regard, the present studies provide some fundamental insights into their structure-activity relationships. We found that unlike HG, which promotes FXR expression, its saponin (*i.e.*, glycosylated) counterparts, hederacoside C and α-hedrin, were without effect. This likely reflects the fact that the glycosylated forms of the molecule are more water soluble and therefore unable to cross the cell membrane into the cytosol. These findings are particularly interesting given that PCTs are predominantly found in their glycosylated forms in plants (40). However, the sugar moieties of these molecules can be metabolized by the β-glyscosidase activity of colonic bacteria, including firmicutes, bacteroidetes, and actinobacteria (41), yielding their more membrane-permeable aglycone isoforms (40, 42, 43). This suggests that while plant-derived PCTs are largely membrane impermeable until they reach the colon, deglycosylation by the resident microbiota releases the lipophilic aglycone form of the molecule that can more readily cross the cell membrane to upregulate FXR expression. Thus, it is likely that basal FXR signaling in the colonic epithelium is partly determined by levels of PCTs in the diet, and it is tempting to speculate that the reduced expression of FXR that is associated with the pathogenesis of inflammatory bowel disease and colorectal cancer (44, 45) may be at least partly due to diminished deglycosylation of PCTs secondary to alterations in the microbiota. Indeed, while microbial metabolism is an important aspect to consider, several other factors are also likely to influence the bioavailability of diet-derived PCTs in the colon, including the amounts ingested, extent of metabolism by digestive enzymes, intestinal transit time, and disease status. Further studies in animal models and humans will be required to address these issues so that strategies can be developed to enable optimal delivery of PCTs to the colonic epithelium for targeted induction of FXR expression. Further studies are also required to understand the molecular pathways by which PCTs induce upregulation of FXR expression and how this ultimately affects downstream signaling events, including its association with cofactors such as the retinoid x receptor and its binding to FXR response elements in the nucleus.

In conclusion, our studies have shown that dietary PCTs, typified by HG, upregulate the expression of FXR in colonic epithelial cells. Given the multifaceted roles that FXR plays in the regulation of intestinal and metabolic function, such effects could have important implications for understanding how the diet can impact intestinal health and disease pathogenesis. On the basis of these findings, we propose that PCTs could form the basis for a new class of "FXR-targeted" nutraceuticals for the prevention and treatment of intestinal and metabolic disorders.

Experimental procedures

Cell culture and treatments

T_{84} colonic epithelial cells were cultured in a mixture of Dulbecco's Modified Eagle Medium (DMEM)/Ham's F12 nutrient mixture (1:1) (Sigma-Aldrich), supplemented with 10% fetal bovine serum (FBS) (Gibco), 1% penicillin/streptomycin (P/S, Gibco), and 1% L-Glutamine (Gibco). For qRT-PCR and Western blot analyses, cells were seeded onto 24 mm Millicell-hanging cell culture inserts (MCHT06H48, Millipore, Merck) at a density of 2×10^6 cells/insert. For electrophysiological measurements, 5×10^5 cells were seeded onto 12 mm Millicell-hanging cell culture inserts (CLS3407, Corning, Merck). Cells were cultured on inserts until they

developed the polarized, electrically resistant phenotype of native colonic epithelia, that is, when TEER plateaued, as measured in $\Omega.cm^2$ using an EVOM2 Voltohmmeter (World Precision Instruments). The synthetic selective agonist GW4064 (Tocris, Bio-Techne) and natural BA CDCA (Sigma-Aldrich) were used to activate FXR (46). The following compounds of interest were investigated for their effects on FXR signaling: HG (Toronto Research Chemicals), OA (Sigma-Aldrich), hederacoside C (Sigma-Aldrich), and α-hederin (Toronto Research Chemicals). Prior to treatment, T_{84} cell monolayers were equilibrated in serum-free medium for 24 h and were bilaterally treated with FXR agonists, a compound of interest or a combination of both for 1 to 48 h as stated. TEER was measured pretreatment and posttreatment using an EVOM2 Voltohmmeter.

Ex vivo *human tissues*

Macroscopically normal colonic tissue biopsies were obtained from the proximal colon of adult patients undergoing routine surveillance colonoscopy in Beaumont Hospital. Statement of Ethical Approval: Patients agreed to participate by written informed consent and this study was approved by Beaumont Hospital Medical Ethics Committee (approval ID: REC 20/54). The studies in this work abide by the Declaration of Helsinki. Fresh biopsies were washed in PBS supplemented with 1% antibiotics (P/S). Biopsies were then placed in 500 μl of DMEM/Hams F12 media, supplemented with 10% FBS, 1% P/S, and either DMSO (0.05%) or HG (5 μM). Biopsies were incubated for 6 h at 37 °C and 5% CO_2 after which they were removed and placed in RNA later overnight at 4 °C. Total RNA was isolated and purified from samples using RNeasy Plus Mini Kits from QIAGEN, as per manufacturers protocol. Samples underwent DNase treatment, reverse transcription, and polymerase chain reaction as described below.

Ex vivo *murine EDMs*

Crypts from murine proximal colonic tissue were isolated with collagenase type I solution (2 mg/ml, Invitrogen) along with gentamicin (50 μg/ml, Life Technologies) and were incubated at 37 °C in 5% CO_2. During incubation, the collagenase type I solution was resuspended until the intestinal epithelial crypts had dislodged from the tissue. The crypts were then filtered through a 70-μm cell strainer (Thermo Fisher Scientific) and washed with medium composed of DMEM/Ham F-12 with Hepes (Sigma-Aldrich), 10% FBS (Sigma), 1% P/S (Life Technologies), and 1% glutaMAX (Life Technologies). Filtered epithelial units were recovered by centrifugation at 200*g* for 5 min and suspended in the basement membrane matrix Matrigel (Corning) before plating and incubation. For culture, 50% conditioned medium (prepared from L-WRN cells synthesizing Wnt3a, R-spondin, and Noggin, a gift from Dr T. Stappenbeck) with 10 μM of ROCK inhibitor (Y27632) (Selleckchem) and 10 μM of TGF-β inhibitor (Y27632) (Selleckchem) was added (47–49).

To prepare EDMs, single cells were generated by trypsinizing the enteroids for 4 min with 0.025% trypsin (Life Technologies). 2×10^5 cells were seeded onto 6.5 mm Transwells (Costar, Corning) that were previously coated with diluted Matrigel (1:40 in PBS, Life Technologies). The EDMs were differentiated in 5% conditioned medium diluted in advanced DMEM/Hams F12 medium (Life Technologies), with R-spondin and Noggin, 20% FBS, 0.1% glutaMAX, and 10 μM of ROCK inhibitor. Following 24 h of differentiation, EDMs were treated according to the following experimental design: bilateral pretreatment with HG (5 μM, Toronto Research Chemicals) for 1 h followed by bilateral treatment with GW4064 (5 μM, Tocris) for 24 h, each agent singly or a vehicle control. Statement of Ethical Approval: Studies on murine enteroids were carried out with ethical approval from the Institutional Review Board of the University of California San Diego.

FXR-luciferase reporter assay

The FXR-luciferase reporter stable cell line was purchased from Caltag Medsystems at passage 4. This cell line was created by cotransfection of HepG2 cells with an FXR-luciferase reporter vector and a hygromycin expression vector. Cells which were hygromycin resistant were then isolated and tested for GW4064-induced luciferase activity. The cells which exhibited the highest induction of luciferase activity were selected and propagated to produce the FXR-luciferase reporter stable cell line. FXR-luciferase reporter cells were cultured in DMEM high glucose medium (Sigma-Aldrich), supplemented with 5% FBS, 1% P/S, and 100 μg/ml hygromycin (5% CO_2 at 37 °C). Twenty four hours prior to treatment, FXR reporter cells were seeded in 96-well white walled clear bottomed plates at a seeding density of 2×10^4 cells/100 μl. Treatments were added at the appropriate concentration for stated length of time. Posttreatment, media was removed by aspiration and 100 μl of PBS was added to each well. PBS was aspirated and 25 μl of 1× luciferase lysis buffer (Signosis) was added to each well. Cells were incubated in lysis buffer for 15 min at room temperature in the dark with gentle agitation. Fifty microliters of luciferase substrate (Signosis) were added to each well. Plates were then read immediately using a Victor microplate spectrophotometer (Perkin Elkins) set at luminescence with 10 s integration.

Electrophysiological measurements

For measurements of transepithelial chloride (Cl^-) secretion, T_{84} cell monolayers were mounted in Ussing chambers (aperture = 1.12 cm^2) and bathed in Ringer's solution containing (in mmol/l): 140 Na^+, 5.2 K^+, 1.2 Ca^{2+}, 0.8 Mg^{2+}, 119.8 Cl^-, 25 HCO_{3-}, 2.4 H_2PO_{4-}, and 10 D-glucose at 37 °C and gassed with 95% O_2, 5% CO_2. T_{84} cell monolayers were voltage-clamped to zero potential difference and monitored for changes in short circuit current (I_{sc}), which in T_{84} cells has been shown to be wholly due to electrogenic Cl^- secretion (50–53). After an equilibration period of 20 min, cells were apically stimulated with the cAMP- secretagogue, FSK (10 μM) (Sigma-Aldrich), and changes in I_{sc} were monitored using Acquire and Analyse software (Physiologic Instruments).

Western blotting

Posttreatment, plates with T_{84} cell monolayers were placed on ice. Cells were washed twice with ice-cold PBS. Hundred microliters of ice-cold lysis buffer containing 10 mM Trizma base, 100 mM NaCl, 1 mM EDTA, 1 mM NaF, 1 mM EGTA, 20 mM $Na_4P_2O_7$, 2 mM Na_3VO_4, 1% Triton X-100, 10% glycerol, 0.1% SDS, 0.5% sodium deoxycholate, protease, and phosphatase inhibitors were added to each insert, and cells were lysed *in situ* for at least 1 h on ice. Cells were scraped and sonicated using 3 × 3 s pulses to promote lysis and increase protein yield. Lysed cells were then centrifuged (12,000 RPM; 10 min; 4 °C). The supernatant was retained, and debris pellet was discarded. Samples, normalized for protein content, were mixed with an equal volume of 2× laemmli loading buffer (1/1, v/v) (Sigma-Aldrich), boiled for 5 min at 95 °C or 30 min at 37 °C, and loaded onto a 10% or 8% tricine-SDS polyacrylamide gel. After gel electrophoresis, transfer to a PVDF membrane was performed for 1.5 h at 15 V. Immunoblotting was performed with the following antibodies: mouse anti-FXR (1: 1000 dilution; R&D Systems), mouse anti-CFTR clone M3A7 (1: 1000 dilution; Millipore), and mouse anti-VDR (1: 1000 dilution; Santa Cruz). Chemiluminescence was detected using appropriate exposure time on the Amersham Imager 600 (GE Healthcare). Images were captured and protein expression analysis was performed using ImageQuant TL software (GE Healthcare). Protein expression was quantified by densitometry and normalized to β-actin (1: 10,000 dilution; Sigma-Aldrich) as a loading control. Antibodies were prepared in 5% bovine serum albumin in tris-buffered saline with 0.3% tween.

Real-time quantitative PCR

Posttreatment, plates were placed on ice. Cells were washed twice with ice-cold PBS. One microliter of ice-cold PBS was added to each insert, and cells were scraped from the inserts using cell scrapers. Scraped cells in PBS were placed in endotoxin-free tubes. Inserts were washed with an additional 1 ml of ice-cold PBS, and the collected 2 ml of scraped cells were centrifuged (11,000 RPM; 5 min; 4 °C). Total RNA was isolated and purified from samples using RNeasy Plus Mini Kits (QIAGEN), as per the manufacturer's protocol. The RNA then underwent DNase treatment using a commercially available kit from Invitrogen (Catalog # AM1906) to remove genomic DNA contamination. Spectrophotometric measurements were taken to assess RNA quantity and quality. Readings of RNA yield (ng/ml), A260/280 and A260/230, were taken on a Nanodrop 2000 (Thermo Scientific). Reverse transcription was performed on purified RNA (0.75 μg) using the Improm-II Reverse Transcription Kit (Promega; Catalog # A3800), as per manufacturer's protocol. The reverse transcription program was run on a PCR Thermal Cycler machine (DNA Engine, DYAD). qRT-PCR was performed to measure the expression of certain target genes. Complementary DNA (0.75 μg) was pipetted into the wells of a 96-well fast thermal cycling plate (Biosciences). A master mix containing SYBR Green Master Mix (Biosciences), nuclease free water, and 0.5 μM forward and reverse primers (Invitrogen) (Table S1) were added to each well. Amplification was performed using the Applied Biosciences 7500 Fast Real Time PCR Systems (Thermo Fisher Scientific).

Murine EDMs were lysed with RNA Lysis Buffer (Zymo Research), as per the manufacturer's protocol. Lysates were placed in Zymo-Spin IC Columns for washing and purification for proper RNA isolation. Reverse transcription was performed using qScript cDNA supermix (Quantabio). cDNA was used for qRT-PCR using 2× SYBR Green qPCR Master Mix (Bimake).

Enzyme linked immunosorbent assay

FGF-19 protein production from monolayers of T_{84} colonic cells was determined by the use of sandwich ELISA. An antibody pair was used for human FGF-19 from DuoSet ELISA Development System from R&D Systems (Catalog # DY969), and procedure was carried out as per manufacturer's protocol. ELISA result was read using a Victor microplate spectrophotometer (Perkin Elkins).

Statistical analysis

The n number for each experiment refers to the biological replicates. Results are expressed as the mean ± SD for n biological replicates. Statistical analysis of the data was performed using GraphPad Prism 9 software. The following tests were used unless otherwise stated. For comparisons of paired treatments between 2 groups, paired Students t-tests were performed. For comparisons of more than 2 groups, with one factor, one-way ANOVA using the Dunnett's or Tukey's post hoc test was performed. For comparisons of more than 2 groups with two factors, two-way ANOVA with Sidak's post-test were performed. p values of $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$ were considered as statistically significant differences between groups and are represented on figures as *, **, and ***, respectively.

Data availability

All relevant data has been submitted along with this article, with each figure uploaded as a separate PDF file.

Supporting information—This article contains supporting information.

Author contributions—C. M. F., J. S. S., H. S., and S. J. K. conceptualization; C. M. F., J. S. S., A. Q., and S. J. K. methodology; C. M. F., J. S. S., and A. Q. formal analysis; C. M. F., J. S. S., and A. Q. investigation; C. M. F. and S. J. K. writing–original draft; C. M. F., J. S. S., A. Q., N. L.-M., A. O. T., K. E. B., H. S., and S. J. K. writing–review and editing; C. M. F., J. S. S., and A. Q. visualization; C. M. F., N. L.-M., K. E. B., and S. J. K. supervision; C. M. F., K. E. B., H. S., and S. J. K. funding acquisition; J. S. S. validation; A. O. T. and K. E. B. resources.

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Abbreviations—The abbreviations used are: BA, bile acid; CDCA, chenodeoxycholic acid; CFTR, cystic fibrosis transmembrane conductance regulator; EDM, enteroid-derived monolayer; EHC, enterohepatic circulation; FBS, fetal bovine serum; FSK, forskolin; FXR, farnesoid X receptor; HG, hederagenin; OA, oleanolic acid; P/S, penicillin/streptomycin; PCT, pentacyclic triterpene; qRT-PCR, quantitative real-time PCR; VDR, vitamin D receptor.

References

1. Parks, D. J., Blanchard, S. G., Bledsoe, R. K., Chandra, G., Consler, T. G., Kliewer, S. A., *et al.* (1999) Bile acids: natural ligands for an orphan nuclear receptor. *Science* **284**, 1365–1368
2. Makishima, M., Okamoto, A. Y., Repa, J. J., Tu, H., Learned, R. M., Luk, A., *et al.* (1999) Identification of a nuclear receptor for bile acids. *Science* **284**, 1362–1365
3. Chiang, J. Y. L., and Ferrell, J. M. (2019) Bile acids as metabolic regulators and nutrient sensors. *Annu. Rev. Nutr.* **39**, 175–200
4. Ahmad, T. R., Higuchi, S., Bertaggia, E., Hung, A., Shanmugarajah, N., Guilz, N. C., *et al.* (2020) Bile acid composition regulates the manganese transporter Slc30a10 in intestine. *J. Biol. Chem.* **295**, 12545–12558
5. Hegyi, P., Maleth, J., Walters, J. R., Hofmann, A. F., and Keely, S. J. (2018) Guts and gall: bile acids in regulation of intestinal epithelial function in health and disease. *Physiol. Rev.* **98**, 1983–2023
6. Inagaki, T., Choi, M., Moschetta, A., Peng, L., Cummins, C. L., McDonald, J. G., *et al.* (2005) Fibroblast growth factor 15 functions as an enterohepatic signal to regulate bile acid homeostasis. *Cell Metab.* **2**, 217–225
7. Kliewer, S. A., and Mangelsdorf, D. J. (2015) Bile acids as hormones: the FXR-FGF15/19 pathway. *Dig. Dis.* **33**, 327–331
8. Inagaki, T., Moschetta, A., Lee, Y. K., Peng, L., Zhao, G., Downes, M., *et al.* (2006) Regulation of antibacterial defense in the small intestine by the nuclear bile acid receptor. *Proc. Natl. Acad. Sci. U. S. A.* **103**, 3920–3925
9. Stojancevic, M., Stankov, K., and Mikov, M. (2012) The impact of farnesoid X receptor activation on intestinal permeability in inflammatory bowel disease. *Can. J. Gastroenterol.* **26**, 631–637
10. Sorribas, M., Jakob, M. O., Yilmaz, B., Li, H., Stutz, D., Noser, Y., *et al.* (2019) FXR modulates the gut-vascular barrier by regulating the entry sites for bacterial translocation in experimental cirrhosis. *J. Hepatol.* **71**, 1126–1140
11. Song, M., Ye, J., Zhang, F., Su, H., Yang, X., He, H., *et al.* (2019) Chenodeoxycholic acid (CDCA) protects against the lipopolysaccharide-induced impairment of the intestinal epithelial barrier function via the FXR-MLCK pathway. *J. Agric. Food Chem.* **67**, 8868–8874
12. Verbeke, L., Farre, R., Verbinnen, B., Covens, K., Vanuytsel, T., Verhaegen, J., *et al.* (2015) The FXR agonist obeticholic acid prevents gut barrier dysfunction and bacterial translocation in cholestatic rats. *Am. J. Pathol.* **185**, 409–419
13. Liu, H. M., Liao, J. F., and Lee, T. Y. (2017) Farnesoid X receptor agonist GW4064 ameliorates lipopolysaccharide-induced ileocolitis through TLR4/MyD88 pathway related mitochondrial dysfunction in mice. *Biochem. Biophys. Res. Commun.* **490**, 841–848
14. Ceulemans, L. J., Verbeke, L., Decuypere, J. P., Farre, R., De Hertogh, G., Lenaerts, K., *et al.* (2017) Farnesoid X receptor activation attenuates intestinal ischemia reperfusion injury in rats. *PLoS One* **12**, e0169331
15. Fiorucci, S., Mencarelli, A., Cipriani, S., Renga, B., Palladino, G., Santucci, L., *et al.* (2011) Activation of the farnesoid-X receptor protects against gastrointestinal injury caused by non-steroidal anti-inflammatory drugs in mice. *Br. J. Pharmacol.* **164**, 1929–1938
16. Gadaleta, R. M., van Erpecum, K. J., Oldenburg, B., Willemsen, E. C., Renooij, W., Murzilli, S., *et al.* (2011) Farnesoid X receptor activation inhibits inflammation and preserves the intestinal barrier in inflammatory bowel disease. *Gut* **60**, 463–472
17. Mroz, M. S., Keating, N., Ward, J. B., Sarker, R., Amu, S., Aviello, G., *et al.* (2014) Farnesoid X receptor agonists attenuate colonic epithelial secretory function and prevent experimental diarrhoea *in vivo*. *Gut* **63**, 808–817
18. Nevens, F., Andreone, P., Mazzella, G., Strasser, S. I., Bowlus, C., Invernizzi, P., *et al.* (2016) A placebo-controlled trial of obeticholic acid in primary biliary cholangitis. *N. Engl. J. Med.* **375**, 631–643
19. Walters, J. R., Johnston, I. M., Nolan, J. D., Vassie, C., Pruzanski, M. E., and Shapiro, D. A. (2015) The response of patients with bile acid diarrhoea to the farnesoid X receptor agonist obeticholic acid. *Aliment. Pharmacol. Ther.* **41**, 54–64
20. Liu, W., and Wong, C. (2010) Oleanolic acid is a selective farnesoid X receptor modulator. *Phytother. Res.* **24**, 369–373
21. Lu, Y., Zheng, W., Lin, S., Guo, F., Zhu, Y., Wei, Y., *et al.* (2018) Identification of an oleanane-type triterpene hedragonic acid as a novel farnesoid X receptor ligand with liver protective effects and anti-inflammatory activity. *Mol. Pharmacol.* **93**, 63–72
22. Gu, M., Zhao, P., Zhang, S., Fan, S., Yang, L., Tong, Q., *et al.* (2019) Betulinic acid alleviates endoplasmic reticulum stress-mediated nonalcoholic fatty liver disease through activation of farnesoid X receptors in mice. *Br. J. Pharmacol.* **176**, 847–863
23. Yu, M., Liu, J., Li, L., Xu, H., Xing, Y., Zhao, Y., *et al.* (2016) Pharmacokinetic parameters of three active ingredients hederacoside C, hederacoside D, and a-hederin in Hedera helix in rats. *J. Sep. Sci.* **39**, 3292–3301
24. Zeng, J., Huang, T., Xue, M., Chen, J., Feng, L., Du, R., *et al.* (2018) Current knowledge and development of hederagenin as a promising medicinal agent: a comprehensive review. *RSC Adv.* **8**, 24188–24202
25. Sieben, A., Prenner, L., Sorkalla, T., Wolf, A., Jakobs, D., Runkel, F., *et al.* (2009) Alpha-hederin, but not hederacoside C and hederagenin from Hedera helix, affects the binding behavior, dynamics, and regulation of beta 2-adrenergic receptors. *Biochemistry* **48**, 3477–3482
26. Sonne, D. P. (2021) Mechanisms in endocrinology: FXR signalling: a novel target in metabolic diseases. *Eur. J. Endocrinol.* **184**, R193–R205
27. Moses, T., Papadopoulou, K. K., and Osbourn, A. (2014) Metabolic and functional diversity of saponins, biosynthetic intermediates and semi-synthetic derivatives. *Crit. Rev. Biochem. Mol. Biol.* **49**, 439–462
28. Jager, S., Trojan, H., Kopp, T., Laszczyk, M. N., and Scheffler, A. (2009) Pentacyclic triterpene distribution in various plants - rich sources for a new group of multi-potent plant extracts. *Molecules* **14**, 2016–2031
29. Furtado, N. A. J. C., Pirson, L., Edelberg, H., Miranda, L. M., Loira-Pastoriza, C., Preat, V., *et al.* (2017) Pentacyclic triterpene bioavailability: an overview of in vitro and in vivo studies. *Molecules* **22**, 400
30. Liu, B. X., Zhou, J. Y., Li, Y., Zou, X., Wu, J., Gu, J. F., *et al.* (2014) Hederagenin from the leaves of ivy (Hedera helix L.) induces apoptosis in human LoVo colon cells through the mitochondrial pathway. *BMC Complement. Altern. Med.* **14**, 412
31. Fang, L., Liu, M., and Cai, L. (2019) Hederagenin inhibits proliferation and promotes apoptosis of cervical cancer CaSki cells by blocking STAT3 pathway. *Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi* **35**, 140–145
32. Wang, K., Liu, X., Liu, Q., Ho, I. H., Wei, X., Yin, T., *et al.* (2020) Hederagenin potentiated cisplatin- and paclitaxel-mediated cytotoxicity by impairing autophagy in lung cancer cells. *Cell Death Dis.* **11**, 611
33. Lee, C. W., Park, S. M., Zhao, R., Lee, C., Chun, W., Son, Y., *et al.* (2015) Hederagenin, a major component of Clematis mandshurica Ruprecht root, attenuates inflammatory responses in RAW 264.7 cells and in mice. *Int. Immunopharmacol.* **29**, 528–537
34. Zhou, D., Jin, H., Lin, H. B., Yang, X. M., Cheng, Y. F., Deng, F. J., *et al.* (2010) Antidepressant effect of the extracts from Fructus Akebiae. *Pharmacol. Biochem. Behav.* **94**, 488–495

35. Zhao, J., Xu, F., Huang, H., Gu, Z., Wang, L., Tan, W., *et al.* (2013) Evaluation on anti-inflammatory, analgesic, antitumor, and antioxidant potential of total Saponins from Nigella glandulifera seeds. *Evid. Based Complement. Alternat. Med.* **2013**, 827230
36. Chandra, S., and Rawat, D. S. (2015) Medicinal plants of the family Caryophyllaceae: a review of ethno-medicinal uses and pharmacological properties. *Integr. Med. Res.* **4**, 123–131
37. Li, Q., Hu, J., Nie, Q., Chang, X., Fang, Q., Xie, J., *et al.* (2021) Hypoglycemic mechanism of polysaccharide from Cyclocarya paliurus leaves in type 2 diabetic rats by gut microbiota and host metabolism alteration. *Sci. China Life Sci.* **64**, 117–132
38. Yang, F., Gong, J., Wang, G., Chen, P., Yang, L., and Wang, Z. (2016) Waltonitone inhibits proliferation of hepatoma cells and tumorigenesis via FXR-miR-22-CCNA2 signaling pathway. *Oncotarget* **7**, 75165–75175
39. Chen, J. C., Chang, Y. S., Wu, S. L., Chao, D. C., Chang, C. S., Li, C. C., *et al.* (2007) Inhibition of Escherichia coli heat-labile enterotoxin-induced diarrhea by Chaenomeles speciosa. *J. Ethnopharmacol.* **113**, 233–239
40. Yu, K., Chen, F., and Li, C. (2012) Absorption, disposition, and pharmacokinetics of saponins from Chinese medicinal herbs: what do we know and what do we need to know more? *Curr. Drug Metab.* **13**, 577–598
41. Dabek, M., McCrae, S. I., Stevens, V. J., Duncan, S. H., and Louis, P. (2008) Distribution of beta-glucosidase and beta-glucuronidase activity and of beta-glucuronidase gene gus in human colonic bacteria. *FEMS Microbiol. Ecol.* **66**, 487–495
42. Cao, W. Y., Wang, Y. N., Wang, P. Y., Lei, W., Feng, B., and Wang, X. J. (2015) Ardipusilloside-I metabolites from human intestinal bacteria and their antitumor activity. *Molecules* **20**, 20569–20581
43. Foti, M. E., and Pies, R. W. (1986) Lithium carbonate and tardive dyskinesia. *J. Clin. Psychopharmacol.* **6**, 325–326
44. Gadaleta, R. M., Garcia-Irigoyen, O., and Moschetta, A. (2017) Bile acids and colon cancer: is FXR the solution of the conundrum? *Mol. Aspects Med.* **56**, 66–74
45. Modica, S., Murzilli, S., Salvatore, L., Schmidt, D. R., and Moschetta, A. (2008) Nuclear bile acid receptor FXR protects against intestinal tumorigenesis. *Cancer Res.* **68**, 9589–9594
46. Maloney, P. R., Parks, D. J., Haffner, C. D., Fivush, A. M., Chandra, G., Plunket, K. D., *et al.* (2000) Identification of a chemical tool for the orphan nuclear receptor FXR. *J. Med. Chem.* **43**, 2971–2974
47. Sato, T., Stange, D. E., Ferrante, M., Vries, R. G., Van Es, J. H., Van den Brink, S., *et al.* (2011) Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. *Gastroenterology* **141**, 1762–1772
48. Sato, T., Vries, R. G., Snippert, H. J., van de Wetering, M., Barker, N., Stange, D. E., *et al.* (2009) Single Lgr5 stem cells build crypt-villus structures *in vitro* without a mesenchymal niche. *Nature* **459**, 262–265
49. Miyoshi, H., and Stappenbeck, T. S. (2013) *In vitro* expansion and genetic modification of gastrointestinal stem cells in spheroid culture. *Nat. Protoc.* **8**, 2471–2482
50. Cartwright, C. A., McRoberts, J. A., Mandel, K. G., and Dharmsathaphorn, K. (1985) Synergistic action of cyclic adenosine monophosphate- and calcium-mediated chloride secretion in a colonic epithelial cell line. *J. Clin. Invest.* **76**, 1837–1842
51. Dharmsathaphorn, K., Mandel, K. G., Masui, H., and McRoberts, J. A. (1985) Vasoactive intestinal polypeptide-induced chloride secretion by a colonic epithelial cell line. Direct participation of a basolaterally localized Na+, K+, Cl- cotransport system. *J. Clin. Invest.* **75**, 462–471
52. Mandel, K. G., McRoberts, J. A., Beuerlein, G., Foster, E. S., and Dharmsathaphorn, K. (1986) Ba2+ inhibition of VIP- and A23187-stimulated Cl- secretion by T84 cell monolayers. *Am. J. Physiol.* **250**, C486–C494
53. Weymer, A., Huott, P., Liu, W., McRoberts, J. A., and Dharmsathaphorn, K. (1985) Chloride secretory mechanism induced by prostaglandin E1 in a colonic epithelial cell line. *J. Clin. Invest.* **76**, 1828–1836