PPARα-UGT axis activation represses intestinal FXR-FGF15 feedback signalling and exacerbates experimental colitis

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Bile acids play a pivotal role in the pathological development of inflammatory bowel disease (IBD). However, the mechanism of bile acid dysregulation in IBD remains unanswered. Here we show that intestinal peroxisome proliferator-activated receptor α (PPARα)-UDP-glucuronosyltransferases (UGTs) signalling is an important determinant of bile acid homeostasis. Dextran sulphate sodium (DSS)-induced colitis leads to accumulation of bile acids in inflamed colon tissues via activation of the intestinal peroxisome PPARα-UGTs pathway. UGTs accelerate the metabolic elimination of bile acids, and thereby decrease their intracellular levels in the small intestine. Reduced intracellular bile acids results in repressed farnesoid X receptor (FXR)-FGF15 signalling, leading to upregulation of hepatic CYP7A1, thus promoting the de novo bile acid synthesis. Both knockout of PPARα and treatment with recombinant FGF19 markedly attenuate DSS-induced colitis. Thus, we propose that intestinal PPARα-UGTs and downstream FXR-FGF15 signalling play vital roles in control of bile acid homeostasis and the pathological development of colitis.
Bile acids are involved in nascent bile formation, cholesterol elimination and intestinal absorption of lipids and lipid-soluble molecules. Moreover, bile acids are increasingly recognized as signalling molecules in regulating energy homeostasis, inflammation and liver regeneration. However, bile acids, some of which are toxic and can induce apoptotic and necrotic cell death, play important roles in promoting the development of diseases such as the well-defined cholestatic liver disease. Thus, the concentration of bile acids must be strictly regulated to ensure a homeostatic state.

Exposure of cells of the gastrointestinal (GI) tract to repeated high levels of bile acids is a vital risk factor for inflammatory bowel disease (IBD) and GI cancer. Studies from both experimental animal models and clinical patients indicated that the levels of bile acids are increased in the conditions of IBD and colon cancer. The accumulation of taurocholic acid in the GI tract can aggravate colitis via regulating the compositions of intestinal flora, providing a potential mechanism that explains the high prevalence of IBD and colon cancer in the populations with western lifestyle and diet. Moreover, exposure of the colorectal epithelium to high physiological concentrations of bile acids is a major aetiological factor in colorectal carcinogenesis. Together, these lines of evidences strongly indicate that the long continuous activation of liver CYP7A1 and thus the increased intracellular bile acids, FXR is activated to induce protective gene expression circuits against bile acid toxicity in the liver and FGF19 (FGF15 in rodents) that binds with liver machinery is dysregulated and whether it is involved in pathological development of IBD remains to be answered. FXR, a ligand-activated transcriptional factor and thus its activation regulated to ensure a homeostasis state.

In this study, we show that the activation of peroxisome proliferator-activated receptor α (PPARα)-UDP-glucuronosyltransferases (UGTs) axis reduces the intracellular levels of bile acids in the intestine, which compromises intestinal FXR-FGF15 signalling. This dysregulated signalling machinery leads to the continuous activation of liver CYP7A1 and thus the increased de novo synthesis of bile acids, and finally the accumulation of bile acids in the inflamed colon tissues. We further reveal that the intestinal PPARα-UGTs axis upstream of FXR-FGF15 pathway is an important determinant in the pathological development of dextran sulphate sodium (DSS)-induced colitis.

**Results**

**Colitis induces bile acids accumulation in the colon.** Chronic DSS-induced murine colitis model was characterized by mucosal oedema, crypt distortion, a high level of leukocyte and polymorphonuclear infiltration, and dramatically increased expression of proinflammatory cytokines (Supplementary Fig. 1). To assess how the overall bile acid profile was affected, bile acid composition analysis was carried out in different compartments of mice. A panel of bile acids in the liver, small intestine, colon, serum, urine and faeces of normal and DSS mice were detected (Fig. 1). After analysing the individual bile acids in multiple compartments, a previously uncharacterized dysregulation feature of bile acids was identified in colitis. Compared with normal mice, the bile acid pool size was significantly increased in mice of DSS-induced colitis (Fig. 1a), which suggests an enhanced de novo synthesis of bile acids. A compartment-specific dysregulation pattern of bile acids was found. In general, the total levels of bile acids were lower in the serum in mice with colitis (Fig. 1b), while higher levels were observed in the gall bladder, small intestine, colon and faeces (Fig. 1c-e). In the small intestine, multiple unconjugated bile acids, including Chenodeoxycholic acid (CDA), deoxycholic acid (DCA), cholic acid (CA), Ursodeoxycholic acid (UDCA), HCA, and β-muricholic acid (β-MCA), were decreased while the conjugated bile acids increased in colitis mice (Fig. 1f). Of particular interest, the inflamed colon showed a much higher concentration for most of bile acid species (including conjugates and free bile acids), supporting a specific intracellular accumulation of toxic bile acid species in the inflamed colon tissues.

**Hepatic CYP7A1 is upregulated in DSS-induced colitis.** The result of increased bile acid pool size suggests an increased de novo synthesis of bile acids. The regulation pattern of hepatic CYP7A1, the rate-controlling enzyme in the de novo synthesis of bile acids, was thus examined. As expected, the hepatic expression of Cyp7a1 mRNA and protein content was significantly increased in colitis compared with that in control mice (Fig. 2a,b). In contrast, the hepatic expression of Cyp8b1, Cyp7b1 and Cyp27a1 mRNAs was not significantly affected (Fig. 2a), consistent with the similar CA levels observed in the liver of the two groups. Little liver damage and inflammation was found in DSS-induced colitis mice (Supplementary Fig. 2a-d). Hepatic Hnf4α and Lrh-1 were proposed to function as important transcription factors in controlling bile acid synthesis and in the induction of Cyp7a1 by transactivating Cyp7a1 promoter activity. Unexpectedly, both Hnf4α and Lrh-1 mRNA expression were markedly decreased in the liver of colitis mice (Supplementary Fig. 2e). These results indicate that the upregulation of hepatic Cyp7a1 is unlikely attributed to the activation of positive regulating signals in the liver.

**Intestinal FXR-FGF15 signalling is compromised in colitis.** As intestinal FXR-FGF15 signalling is the major determinant of hepatic CYP7A1 (refs 18,19), the possibility that intestinal FXR-FGF15 signalling is dysregulated in colitis was explored. No significant change was detected in the ileal Fxr mRNA (Fig. 2c) and protein content (Fig. 2g). However, the mRNA expression of FXR target genes, including Fgf15 and Shp (Fig. 2d,e), decreased markedly in colitis mice. Immunohistochemical staining also indicated a significantly decreased protein level of FGF15 in the ileum section (Fig. 2h). Of interest, the expression of Fgfr4 in the liver of colitis group was also decreased, while β-Klotho remained unchanged (Fig. 2f). These data indicate a decrease in the intestinal negative feedback pathway of bile acids synthesis in DSS-induced colitis mice.

**Glcuronidation of bile acids is facilitated in colitis.** FXR is a ligand-activated transcriptional factor and thus its activation is largely dependent on the intracellular level of its ligands.
Colitis resulted in a significantly decreased level of multiple FXR ligands, including CDCA, DCA and CA, in the small intestine where they function to activate the FXR-FGF15 signalling (Fig. 1f). We thus reasoned that the decreased level of intracellular ligands of FXR in the small intestine might be one of the major mechanisms underlying the compromised FXR-FGF15 signalling in colitis. For this reason, the mechanisms responsible for the reduced intracellular levels of FXR ligands in the small intestine were investigated.

The expression of transporters responsible for absorption of bile acids was measured in the distal ileum. Both apical and basal transporters, including Asbt, Ostx and Ostf1, and the intracellular bile acids binding protein Ibabp, remained largely unchanged (Supplementary Fig. 3), suggesting that the reduction of bile acids in the enterocytes could be due to a reduced bile acid absorption.

**Figure 1 | DSS-induced colitis disrupts bile acids homeostasis.** (a) Bile acid pool analysis. The bile acid pool size was analysed by measuring total bile acids in the whole enterohepatic system, including the liver, gall bladder and the entire small intestine and its contents, and the values were normalized by body weight. (b–e) Total amount of bile acids in individual compartments. (f) Compartmental individual bile acid profiles analysed by a UFLC-Triple-time of flight/MS analysis in the control and colitis mice. Results are mean ± s.e.m of six mice, *P<0.05, **P<0.01 versus normal control, Student’s t-test.

CA, cholic acid; LCA, lithocholic acid; UDCA, ursodeoxycholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; MCA, muricholic acid; T, tauro-conjugated species; G, glycine-conjugated species.
intracellular FXR ligands is unlikely caused by bile acids malabsorption. In addition to cellular uptake capacity, metabolic elimination is another important determinant of the intracellular levels for both endogenous and exogenous agents. Quantitative analysis of bile acids species showed that the glucuronidation metabolites of bile acids were dramatically enhanced in both the faeces and urine (Fig. 3a,b). We thus checked whether the expression and activity of UGTs in the small intestine are elevated. The hepatic expression of UGts has been previously shown to play an important role in the metabolic elimination of bile acids\(^\text{20–22}\). However, very little is known about the role of intestinal UGTs in determining the homeostasis of bile acids. UGT1A3 and UGT2B4 were found to be the dominant UGT isoforms involved in the glucuronidation of bile acids in humans\(^\text{20,23}\); however, the involvement of several other isoforms, including UGT1A4, UGT2B7, UGT2A1 and UGT2A2, has recently been reported\(^\text{24,25}\). These findings are in line with the general view about the largely overlapping property of UGTs in metabolism. Moreover, the exact UGT isoforms in the metabolism of bile acids in mice remains elusive. Thus, the major UGT isoforms expressed in the small intestine of mice\(^\text{26}\) were determined. The mRNA levels for most of the Ugt isoforms, including Ugt1a6, Ugt1a7, Ugt2b24 and Ugt2b35, were significantly upregulated in the small intestine of mice with DSS-induced colitis (Fig. 3c). Consistent with the mRNA data, the enzymatic activities for all the tested substrates in the small intestine of colitis mice were dramatically increased (Fig. 3d). In particular, the intestinal enzymatic activity of CDCA

**Figure 2 | The intestinal FXR-FGF15 signalling is compromised in colitis.** (a) The hepatic expression of enzymes involved in bile acid biosynthetic pathway. (b) Western blot analysis of CYP7A1 protein content in the liver. (c-e) Expression of Fxr, Fgf15 and Shp mRNAs in the distal ileum. (f) Expression of Fgr4 and β-Klotho mRNAs in the liver. (g) Immunohistochemistry staining and quantitative analysis of FXR protein content in the distal ileum (scale bar, 50 μm). (h) Immunohistochemistry staining and quantitative analysis of FGF15 protein content in the distal ileum (scale bar, 50 μm). Results of quantitative analysis values are expressed as mean ± s.e.m. (n = 6) and are plotted as fold change. *P < 0.05, **P < 0.01 versus normal control, Student’s t-test.
glucuronidation was enhanced by fourfold (Fig. 3d and Supplementary Table 1). This result strongly supports a facilitated metabolic elimination of potential FXR ligands from the intestinal epithelium and thus a significant reduction of intracellular levels of CDCA, CA and DCA, all of which are potent FXR ligands in mice. Moreover, human intestinal S9 fraction possessed a much stronger enzyme activity towards the glucuronidation of CDCA (Supplementary Fig. 4), hinting to a more important role of intestinal UGTs in regulating the homeostasis of bile acids in humans.

Of interest, the mRNA levels and enzyme activity of some UGTs in the liver were also slightly increased (Supplementary Fig. 5a,b), consistent with the slightly increased glucuronidation products of bile acids in the urine of colitis mice. It was somewhat unexpected to find that the enzymatic expression and activity of multiple UGTs in the colon of colitis mice were also significantly enhanced by fourfold (Fig. 3d and Supplementary Table 1). This result strongly supports a facilitated metabolic elimination of potential FXR ligands from the intestinal epithelium and thus a significant reduction of intracellular levels of CDCA, CA and DCA, all of which are potent FXR ligands in mice. Moreover, human intestinal S9 fraction possessed a much stronger enzyme activity towards the glucuronidation of CDCA (Supplementary Fig. 4), hinting to a more important role of intestinal UGTs in regulating the homeostasis of bile acids in humans.

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**Figure 3 | Intestinal PPARα-UGTs axis is overactivated in colitis.** (a,b) The quantitative analysis of glucuronidation metabolites of bile acids in faeces and urine. (c,d) Expression and enzyme activity of UGT1A1, UGT1A6, UGT1A7, UGT2B34 and UGT2B35. (e) Expression of Ppara, Acox1 and L-Fabp mRNAs in the small intestine. (f) Immunohistochemistry staining and quantitative analysis of PPARα protein content in the distal ileum (scale bar, 50 μm). (g) The concentration of OEA in the intestine contents of colitis mice, analysed by LC-MS. (h) The mRNA expression of Nape-pld and Faah in the small intestine. Results of mRNA expression are mean ± s.e.m. of six mice. *P < 0.05, **P < 0.01 versus normal control, Student’s t-test. Results of UGT activity were determined towards various substrates in pooled mice S9 (n = 6); data are the means of triplicate determinations. Bile salts species were analysed by the UFLC-Triple-time-of-flight/MS analysis (n = 6).
enhanced (Supplementary Fig. 5c,d), in spite of the dramatic accumulation of multiple bile acids species in the colon tissues. Together, these results suggest that the expression and activity of UGTs and, in particular, in the GI tract are largely enhanced under the condition of colitis, facilitating the metabolic elimination of bile acids.

**PPARα underlies UGTs and bile acid disturbance in colitis.**

Previous studies illustrated that PPARα is an important transcriptional factor in regulating the expression of various UGT enzymes and can also regulate the homeostasis of bile acids by unclear mechanisms. Hence, we assume that PPARα may play a critical role in mediating the dysregulation of bile acids in colitis. The increased mRNA expression of 

**Acox1**

and 

**L-Fabp**

suggests that the PPARα signalling pathway in the small intestine was activated in colitis mice (Fig. 3e). Although the ileal expression of 

**Pparα**

mRNA was not significantly changed, immunohistochemical staining showed a slight increase in its protein expression (Fig. 3e,f). It was reported that oleylethanolamide (OEA) is a natural endogenous lipid that activates PPARα with high potency and exert anorectic actions. Consequently, a prominent rationale is that the activation of PPARα pathway is dependent on the elevated concentration of its endogenous ligands. To this end, we investigated OEA levels by liquid chromatography–mass spectrometry and found a strongly enhanced level in the intestine contents of colitis mice (Fig. 3g). NAPE-PLD (N-acyl phosphatidylethanolamine-selective phospholipase D) is responsible for the biosynthesis of OEA, while FAAH (fatty acid amide hydrolase) is involved in its degradation. Here, the upregulated expression of 

**Nape-pld**

mRNA and the unchanged expression of 

**Faah**

mRNA (Fig. 3h) suggest that the increased level of OEA in colitis mice is likely due to the enhanced biosynthesis. The increased OEA biosynthesis, upregulation of PPARα typical target genes, together with the upregulated expression of 

**Ugts**

suggest an activated PPARα-UGT axis in the intestine of colitis mice. To ascertain whether the PPARα activation can regulate intestinal FXR-FGF15 signalling and the bile acid homeostasis, normal mice were administered with the PPARα agonist Wy-14643. Wy-14643 treatment for 1 week significantly upregulated the mRNAs of typical PPARα target genes 

**Acox1**

and 

**L-Fabp**

in the small intestine (Supplementary Fig. 6a–c) and resulted in a robust enhancement of the intestinal expression of multiple 

**Ugts**

(Fig. 4b). In particular, the enzymatic activity towards the glucuronidation of CDCA in the intestinal S9 fraction of 

**WT**

mice was not significantly changed, 

**Ppara**

-UGTs axis in the regulation of bile acids homeostasis, a cellular pharmacokinetic study of CDCA and its association with FXR-FGF15 (FGF19 in humans) signalling was carried out. In a rat intestinal epithelial cell line IEC-6, treatment with Wy-14643 significantly increased the production of CDCA glucuronide (Supplementary Fig. 8a). Moreover, the intracellular exposure level of CDCA was decreased by Wy-14643 treatment, which was closely associated with a significant reduction in activating 

**Fxr-Fgf15**

signalling (Fig. 4j). Moreover, the faecal glucuronides of most bile acid species were much lower in the 

**Ppara-null**

mice than that in WT mice (Supplementary Fig. 8b).

**PPARα-UGTs axis controls FXR-FGF19 signalling in enterocytes.**

To further validate the role of PPARα in regulating FXR-FGF15 signalling, a comparative study between wild-type (WT) mice and 

**Ppara-null**

mice was performed (Supplementary Fig. 8a). In agreement with a recent study, hepatic expression of 

**Cyp7a1**

mRNA in 

**Ppara-null**

mice was much lower than that in WT mice (Fig. 4g). The downregulation of hepatic 

**Cyp7a1**

in 

**Ppara-null**

mice is at least partially due to the increased intestinal upregulation of 

**Fgf15**

(Fig. 4h). In support of a role for PPARα in regulating intestinal UGTs, the intestinal expression of several 

**Ugt**

genes was significantly lower than in WT mice (Fig. 4i). The enzymatic activity towards the glucuronidation of CDCA in the intestinal 

**S9**

fraction of 

**Ppara-null**

mice was lower than that in WT mice (Fig. 4j). Moreover, the faecal glucuronides of most bile acid species were much lower in the 

**Ppara-null**

mice than that in WT mice (Supplementary Fig. 8b).

To provide a translational link to humans, this study was extended to HT29 cells by using OEA and fenofibrate (a specific human PPARα agonist) treatment. The cellular kinetic profiles clearly indicated that the activation of PPARα by OEA and fenofibrate dramatically decreased intracellular level of CDCA, while small interfering RNA (siRNA) silencing of 

**Ugt1a3**

significantly increased CDCA levels (Supplementary Fig. 10a–d). The experiments conducted in primary murine intestinal epithelial cells (PMIECs) further validated these findings. Both OEA and Wy-14643 treatment significantly promoted the metabolic elimination of CDCA and CA in PMIECs, as judged by decreased intracellular concentrations of CDCA and CA, and increased glucuronides of them (Fig. 5a,b,d,e). Consistently, the expression of FXR target genes 

**Fgf15**

and 

**Shp**

was markedly reduced on OEA and Wy-14643 treatment (Fig. 5c,f), indicating a repressed FXR activation as a result of PPARα activation.
Figure 4 | PPARα controls intestinal FXR-FGF15 and bile acid homeostasis. (a-f) The mice were treated with Wy-14643 (50 mg kg⁻¹, i.g., once a day) for 1 week. Results are mean ± s.e.m. of eight mice. *P<0.05, **P<0.01 versus control, Student’s t-test. (a) The enzymatic activity of UGT isozymes towards the glucuronidation of CDCA. The mice S9 in the distal ileum were a pool of eight individuals. (b) The mRNA expression of Ugt1a1, Ugt1a3, Ugt1a7, Ugt2b34 and Ugt2b35. (c) The ileum expression of Fxr and Fgf15 in control and Wy-14643-treated mice. (d,e) The hepatic expression and protein content of CYP7A1 in control and WY-14643-treated mice. (f) Small intestinal bile acid profiles of control and Wy-14643-treated mice using UFLC-triple-time of flight analysis. (g-j) Comparative study in Ppara-null and WT mice; results of quantitative analysis values are expressed as mean ± s.e.m. (n = 6) and are plotted as fold change. *P<0.05, **P<0.01 versus WT, Student’s t-test. (g) The hepatic expression of Cyp7a1, Fgf15, Fgfr4 and Shp in WT and Ppara-null (Ppara⁻/⁻) mice. (h) Expression of Fgf15, Shp and Fxr mRNAs in the ileum. (i,j) The mRNA expression and activity of UgtS in control and Ppara-null mice.
substrate of UGTs and thus is not glucuronidated in HT29 cells. Together, results from the in vitro cellular study provide direct evidence supporting a pivotal role for PPARα and UGTs in determining the intestinal FXR-FGF15 signalling via controlling the intracellular disposition of FXR ligands.

Results obtained from the study in HT29 cells suggest that disruption of the PPARα-UGTs axis can also compromise the intestinal FXR-FGF19 signalling in humans. To develop a translational link to humans, possible dysregulation of PPARα-UGTs and FXR-FGF19 signalling in the colon biopsies from IBD

Figure 5 | PPARα-UGTs axis controls FXR-FGF15 signalling in enterocytes. (a-f) PMIECs were incubated with 50 μM of CA or CDCA in the absence or presence of PPARα agonists. (a-c) The relative levels of glucuronidation metabolites in the media and the intracellular concentration of FXR ligands and the expression of Fgf15 in PMIECs under treatment with OEA. (d-f) The relative levels of glucuronidation metabolites in the media and the intracellular concentration of FXR ligands, and the expression of Fgf15 in PMIECs under the treatment of Wy-14643. (g-i) The relative levels of glucuronidation metabolites in the media and intracellular concentrations of FXR ligands, and the expression of FGF19 in HT29 cells under the treatment of fenofibrate. (j-o) The relative levels of glucuronidation metabolites in the media and intracellular concentration of FXR ligands, and the expression of FGF19 of control and UGT1A3 siRNA-transfected HT29 cells. Data are the mean ± s.e.m. of three independent experiments and are plotted as fold change. *P<0.05, **P<0.01 versus control, Student’s t-test.
patients was analysed. In line with data obtained from the DSS-induced colitis mice, the PPARγ target genes, including ACOX1 and multiple UGTs, in particular the UGT1A3, were significantly higher, while expression of the FXR target genes, including FGF19 and SHP, were significantly lower than that in the healthy control samples (Supplementary Fig. 11a–c). These results indicate that PPARγ-UGTs signalling is overactivated and may repress the FXR-FGF19 signalling in IBD patients.

**PPARγ-FGF15 signalling controls DSS-induced colitis.** The present results indicated that the PPARγ-FGF15 signalling has a pivotal role in determining bile acid homeostasis under both physiological conditions and in DSS-induced colitis. To determine whether dysregulation of PPARγ-FGF15 signalling is involved in the pathological development of DSS-induced colitis, mice were treated with PPARγ agonist Wy-14643 alone or together with DSS. Although Wy-14643 alone did not induce any inflammatory change in the colon, it markedly enhanced DSS-induced colitis. In a preliminary study, we found that Wy-14643 together with a relatively low dose of DSS at 2% resulted in a high mortality rate and most of the mice could not survive through the three cycles of DSS treatment. Thus, we designed an experimental protocol of 7 days DSS treatment with 3 days washout to determine the influence of PPARγ activation in DSS-induced colitis. By use of this protocol, DSS alone induced a relatively minor extent of inflammatory damage and less evident upregulation of UGTs (Supplementary Fig. 12c), as compared with the chronic DSS-induced colitis (Fig. 3c). Wy-14643 co-treatment with DSS resulted in a more dramatic decrease in body weight and colon length (Fig. 3d,b), more severe inflammatory damage (Fig. 3c) and higher extent of imbalance between proinflammatory and anti-inflammatory cytokines (Supplementary Fig. 12a), when compared with DSS treatment alone. Wy-14643 treatment significantly upregulated the expression of Ppara and its target genes in the ileum, including Acox-1, L-Fabp and multiple Ugt isoform genes (Supplementary Fig. 12b,c). Consistently, the ileum expression of FXR target genes Fgf15 and Shp, and hepatic expression of Fgfr4 and Shp were further decreased (Fig. 6d,e), leading to more upregulated expression of hepatic Cyp7a1 and increased bile acid pool size in the Wy-14643 and DSS co-treated mice (Fig. 6e,f).

To further validate the role of PPARγ in colitis, a comparative study of DSS-induced colitis was performed in WT and Ppara-null mice. Ppara-null mice were less susceptible to chronic DSS-induced colitis, as judged by less decrease in body weight, longer colon length and reduced inflammatory tissue damage, when compared with WT mice (Fig. 7a–c). The ileum expression of FXR target gene L-Fabp and multiple Ugt isoform genes, including Ugt1a1, Ugt1a6 and Ugt1a7 in the Ppara-null mice, was significantly lower than that in the WT littermates (Fig. 7d,e). Consequently, the ileum expression of FXR target genes Fgf15 and Shp was significantly higher, while the hepatic expression of Cyp7a1 was significantly lower in the Ppara-null mice than that in the WT littermates (Fig. 7f,g).

As the reduced ileum secretion of FGF15 is an important determinant in causing dysregulation of bile acids in DSS-induced colitis, the possibility that FGF15 supplementation could ameliorate DSS-induced colitis and restore bile acid homeostasis was investigated. As it was previously reported that recombinant human FGF19 is effective in regulating bile acid homeostasis in mice32, DSS-induced colitis mice were treated with recombinant human FGF19 at a dose of 25 μg kg⁻¹ intraperitoneally. Chronic treatment of FGF19 markedly attenuated DSS-induced colitis, as revealed by a significant increase in the ability to regain weight, longer colon length and improved inflammatory tissue damage (Fig. 8a–c). Moreover, FGF19 treatment significantly restored hepatic expression of Fgfr4 and Shp, and in particular, the hepatic expression of Cyp7a1, and the bile acid pool size returned to normal levels (Fig. 8d–g). Together, these results suggest that reduced secretion of FGF15 is an important factor in facilitating pathological development of DSS-induced colitis and

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**Figure 6 | Wy-14643 exacerbates DSS-induced colitis.** (a) Body weight. (b) Colon length. (c) Histological assessment (scale bar, 100 μm). (d) Ileum expression of Fxr and Fgf15 mRNAs. (e) Hepatic expression of Fgfr4, β-Klotho, Shp and Cyp7a1 mRNAs. (f) Bile acid pool analysis. Results of quantitative analysis values are expressed as mean ± s.e.m. (n = 6) and are plotted as fold change. *P<0.05, **P<0.01 versus DSS, Student’s t-test.
that the chronic FGF19 treatment at a low dose may be a promising strategy for therapy of colitis.

**Discussion**

The current study demonstrated that chronic DSS-induced colitis activates intestinal PPARα-UGTs axis promoting the metabolic elimination of bile acids, endogenous ligands of FXR, in the small intestine. The reduced intracellular level of endogenous FXR ligands results in a repressed activation of intestinal FXR-FGF15 feedback signalling that leads to the upregulation of hepatic CYP7A1 and thereby increasing de novo synthesis of bile acids under conditions of DSS-induced colitis. Our results indicate that the overactivation of the PPARα-UGT axis and consequently the compromised intestinal FXR-FGF15 signalling are key determinants in the pathological development of colitis in the DSS-induced model.

Increased faecal excretion of bile acids is a well-defined pattern in IBD; however, the potential altered disposition of bile acids in other compartments in IBD is less known and controversial in previous studies. Increased faecal excretion but decreased serum levels of bile acids were observed in experimental colitis mice, which conformed to earlier observations in both experimental colitis and IBD patients. The levels of bile acids accumulated in the inflamed colon tissues were found dramatically increased, supporting a potential role for toxic bile acids in potentiating the pathological development of colitis. Although the bile acid pool size increased, it was of interest to note that multiple unconjugated bile acids significantly decreased in the small intestine.

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**Figure 7 | Pparα-null mice are less susceptible to DSS-induced colitis.** (a) Body weight. (b) Colon length. (c) Haematoxylin and eosin staining of colon tissues (scale bar, 100 μm). (d) The mRNA expression of Pparα and L-Fabp in the ileum and liver of WT control and Pparα-null (Pparα −/−) mice. (e) The mRNA expression of Ugts in WT and Pparα −/− mice. (f) The mRNA expression of Fgf15, Shp and Fxr in the ileum. (g) The hepatic expression of Cyp7a1, β-Klotho, Fgfr4 and Shp in WT and Pparα −/− mice. Results of quantitative analysis values are expressed as mean ± s.e.m. (n = 6) and are plotted as fold change. *P < 0.05, **P < 0.01 versus WT, Student’s t-test.
multiple FXR ligands, including CDCA, CA and DCA, in the ileum represent a major mechanism underlying the repressed FXR-FGF15 feedback signalling pathway in colitis. Moreover, the hepatic expression of Fgfr4 was reduced in colitis, which may be related to the reduced FGF15 levels in view that the FGF19 treatment could significantly restore the hepatic expression of Fgfr4. The reduced circulating FGF15 levels and decreased hepatic expression of its receptor Fgfr4 may work together to upregulate Cyp7a1. It was of interest to note that the intracellular accumulation of TβMCA, a recently identified endogenous FXR antagonist, in the ileum is significantly increased in colitis mice. Thus, the possibility cannot be excluded that the increased level of FXR antagonist TβMCA, in addition to a decrease in FXR agonists, may also contribute to compromised ileal FXR-FGF15 signalling in colitis mice. Nevertheless, these findings suggest a facilitated faecal elimination and an increased hepatic de novo synthesis of bile acids in DSS-induced colitis. Such a dysregulation of bile acids in DSS-induced colitis is quite similar to that observed from the use of bile acids sequestrant, which promotes the faecal elimination of bile acids and upregulates the hepatic expression of Cyp7a1. Of particular interest, a recent study suggested a specific enrichment of bile acids in the colon with colestilan treatment. It seems that the dysregulation of bile acids accumulation is quite interest, a recent study suggested a specific enrichment of bile acids in the colon with colestilan treatment. It seems that the dysregulation of bile acids accumulation is quite common in the condition of abnormal bile acids accumulation. Unlike taurine- and glycine-conjugated bile acids, glucuronidated bile acids cannot be reabsorbed and may represent an important detoxification mechanism under the condition of abnormal bile acids accumulation. Previous studies

Figure 8 | FGF19 treatment protects against DSS-induced colitis in mice. DSS-induced chronic colitis mice were treated with recombinant FGF19 at a dose of 25 μg kg⁻¹ per day for the first 14 days per DSS cycle. (a) Body weight recorded during the first DSS cycle. (b) Colon length. (c) Haematoxylin and eosin staining of colon tissues (scale bar, 100 μm). (d) Hepatic Fgfr4, β-klotho, Shp and Fxr mRNA expression. (e) Hepatic Cyp7a1 mRNA expression. (f) Hepatic levels of CYP7A1 protein. (g) Bile acid pool analysis. Results of quantitative analysis values are mean ± s.e.m. of eight mice. *P < 0.05, **P < 0.01 versus DSS, Student’s t-test.
suggested that the hepatic glucuronidation of bile acids may be part of a negative feedback mechanism by which bile acids limit their biological activity and their intracellular level to avoid a pathophysiological accumulation\textsuperscript{23,40}. The present data suggest that the intestinal glucuronidation of bile acids is also an important factor in maintaining the bile acid homeostasis via controlling the intracellular levels of endogenous FXR ligands and consequently the ileal FXR-FGF15 signalling. Although it remains a concern about the exact UGT isoforms involved in the glucuronidation of bile acids in mice, it is reasonable to speculate that multiple UGT isoforms may be involved, considering that multiple UGT isoforms, including UGT1A3, UGT2B4, UGT2B7, UGT2A1 and UGT2A2, were found responsible for bile acid glucuronidation in human beings\textsuperscript{20,24,25}.

Previous studies about the effect of PPAR\textsubscript{α} activation in regulating CYP7A1 and bile acids homeostasis are controversial. Some studies reported that activation of PPAR\textsubscript{α} by fibrates could repress the expression of Cyp7a1 (refs 41,42), which was presumed to be a potential mechanism underlying the side effects of fibrates in causing gall stones in humans. However, other studies indicated that the activation of PPAR\textsubscript{α} by endogenous agonist, such as fatty acid metabolites, could promote Cyp7a1 transcriptional activity\textsuperscript{43} and that the PPAR\textsubscript{α}-specific agonist Wy-14643 increased the bile acid synthesis in mice\textsuperscript{44}. However, none of the previous studies considered the potential influence of PPAR\textsubscript{α} activation in regulating CYP7A1 via the intestinal FXR-FGF15 signalling. The current finding that increased OEA, an endogenous PPAR\textsubscript{α} ligand, and the resultant upregulation of typical PPAR\textsubscript{α} target genes and genes encoding some of the UGTs in the small intestine, indicate an activated PPAR\textsubscript{α}-UGTs axis in colitis mice. Oral administration of the PPAR\textsubscript{α} agonist Wy-14643 largely mimicked the characteristic patterns observed in colitis, including the enhanced UGT expression and activity, repressed intestinal FXR-FGF15 signalling, upregulated hepatic CYP7A1 expression and even the compartmental disposition patterns of bile acids. Moreover, the intestinal FXR-FGF15 feedback signalling was found significantly promoted in the Ppar\textsubscript{α}-null mice. In PMIECs, PPAR\textsubscript{α} activation by Wy-14643 or OEA led to repressed FXR-FGF15 signalling activated by CDCA or CA, an effect that was dependent on the expression and activity of UGTs. Notably, the activation of PPAR\textsubscript{α} by OEA or fenofibrate in the HT29 cell lines led to quite similar findings to that observed in the rodent intestinal cell model. The analysis of colon biopsies further reveals that activation of the PPAR\textsubscript{α}-UGTs axis and repression of FXR-FGF15 signalling also occurs in IBD patients. Moreover, chronic treatment with fenofibrate for three months markedly reduced the serum levels of FGF19 (ref. 45), supporting an important role of PPAR\textsubscript{α} activation in regulating FXR-FGF19 signalling in humans. Together, these results provide a translational link of the findings from the DSS-induced colitis mice to the pathological development of IBD in humans.

Several previous reports suggest a protective effect of PPAR\textsubscript{α} activation against the pathological development of experimental colitis\textsuperscript{46–49}, which are contradictory to the present findings. Herein, a comprehensive evidence is provided that the intestinal PPAR\textsubscript{α} is already overactivated in the chronic DSS-induced colitis model, and that the treatment with PPAR\textsubscript{α} agonist could markedly promote, whereas the knock out of PPAR\textsubscript{α} could significantly retard, the pathological development of DSS-induced colitis. Moreover, the chronic treatment with recombinant FGF19 could significantly attenuate DSS-induced colitis. The discrepancy between the present results and the previous reports may be explained by the use of different animal models and experimental protocols. Notably, previous studies were performed either in acute colitis animal models or in the interleukin-10-deficient mice that spontaneously develop colitis. The protective effects of PPAR\textsubscript{α} activation observed with these mouse models may be explained by the anti-inflammatory effects of PPAR\textsubscript{α} activation. In contrast, the present study showed that prolonged activation of PPAR\textsubscript{α} in the DSS-induced chronic colitis model may actually aggravate the pathological development of colitis via disrupting...
bile acid homeostasis. Previous studies revealed that in a 2,4,6-trinitrobenzenesulfonic acid-induced acute colitis model, most of the UGTs were downregulated. Moreover, the present study revealed that the upregulation of UGTs in the acute DSS colitis-treated mice is less evident than that in the chronic DSS models. These results suggest that the PPARα-UGT axis plays a pivotal role in the regulation of bile acid homeostasis in the colitis model and thus retard pathological development of colitis. Taken together, our results support a pivotal role of prolonged PPARα activation and the resultant bile acid dysregulation in the pathological development of chronic DSS-induced colitis.

In summary, the current study indicates that the intestinal PPARα-UGT axis plays a pivotal role in regulating FXR-FGF15 (FGF19) signalling and bile acid homeostasis. Under conditions of DSS-induced chronic colitis, the prolonged activation of intestinal PPARα-UGT axis promotes the intracellular and extracellular elimination of endogenous FXR ligands in the small intestine which decouples the intestinal FXR-FGF15 feedback signalling from the hepatic (CYP7A1 expression and bile acids de novo synthesis (Fig. 9). Results collected from the present study suggest that restoration of OEA-PPARα-UGT axis and FXR-FGF15 signalling may be of benefits to restore bile acid homeostasis in the colitis model and thus retard pathological development of colitis. Taken together, these results suggest that the regulation of PPARα-UGT axis and the supplementation of recombinant FGF19 may represent promising strategy for the therapy of colitis.

Methods

Materials. OEA was purchased from Santa Cruz Biotechnology (Santa Cruz, CA); DSS (molecular weight = 36,000–50,000 kDa) was obtained from MP Biological (Santa Ana, CA). MPA O-glucuronol and nalone 3-β-D-glucuronol were obtained from Toronto Research Chemicals Inc. (ON, Canada). β-MCA, UDCA, HDCA, G-LCA, G-UDCA, G-DCA, T-β-MCA, T-UDCA, T-HDCA and T-LCA were purchased from Steraloids Inc. (Newport, RI), z-MCA, CA, DCA, CDCA, cholic acid (LCA), G-CDCA, GC-A, T-CDCA, T-DCA, T-CA, dehydrocholic acid and propranolol were purchased from Sigma-Aldrich (St Louis, MO). The anti-UGT1A1, anti-FXR, anti-FGF15 and anti-CYP7A1 antibodies were purchased from Abcam (Cambridge, UK). The anti-glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody was purchased from SunShine Biotechnology (Nanjing, China). The secondary antibodies and recombinant human FGF19 was obtained from Bioworld Technology (St Louis Park, MN). Other reagents, unless mentioned, were obtained from Sigma-Aldrich. RNA extraction of colon biopsies from 8 healthy humans and 13 ulcerative colitis (UC) and 13 Crohn’s disease patients (USA). The mobile phase consisted of 0.1 M ammonium acetate in water and solvent B acetonitrile. A gradient elution of the mobile phase was performed: initial 20% B for 2 min, linear gradient 20–80% B from 2 to 6 min and hold at 80% B for 3.5 min, then quickly returned to initial 20% B in 1.5 min and maintained for a further 2 min for column balance. Due to the poor peak shape, mobile phase was tremendous. The eluates of Glu-CA and Glu-CDCA were expressed as peak area, normalized by dividing peak area of the IS. All experiments were performed in triplicate.

Assessment of mucosal damage and biochemical analysis. Tissue sections (5 μm) were stained with haematoxylin and eosin. Histopathological scoring was performed by an experienced pathologist blinded to the experimental conditions, using an established semiquantitative score ranging from 0 to 6 based on infiltration of inflammatory cells and epithelial damage.

Immunohistochemistry. Tissue specimens were fixed in 10% formalin for 12–24 h, dehydrated and paraffin embedded. Standard immunohistochemical procedures were performed. Tissue sections were incubated with the primary antibody (rabbit polyclonal anti-FXR, 1:100; goat polyclonal anti-FGF15, 1:100; rabbit polyclonal anti-PPARα, 1:200) for 2 h at room temperature, followed by 30 min incubation with the biotinylated secondary antibody (1:400). For negative controls, 1% non-immune serum in PBS replaced the primary antibodies. The immunohistochemistry staining of FXR, FGF15 and PPARα was scored by measuring the integrated optical density at least three views of each slide using Image pro-plus 6.0 software. Data are expressed as mean ± s.e.m. of six mice.

Quantitative reverse transcriptase PCR. Total tissue and cell RNA extraction were performed using the RNAiso Plus reagent (TaKaRa Biotechnology Co., Ltd, Dalian, China) according to the manufacturer’s protocol. Complementary DNA was generated from 500 ng total RNA using SuperScript II Reverse Transcriptase.
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**Western blot analysis.** Tissue extracts were prepared, subjected to SDS-PAGE using 8% gradient (Bio-Rad, Hercules, CA, USA). The blots were stripped and reprobed with antibody to GAPDH (1:2,000) to normalize for protein load. Protein expression was quantified by densitometry using a Microtek ScanMaker 9000 and FujiFilm Multiguage 3 software, and expression data were normalized to levels of the GAPDH loading control.

**UGT activity detection.** Tissues from six to eight mice were pooled and homogenized to make 20% homogenates in the 10 mM phosphate buffer containing 5 mM EDTA. The homogenates were subjected to centrifugation at 9,000 x g before use. The UGT activities were determined towards various substrates in pooled mice S9. A typical incubation mixture contained 50 mM Tris-HCl buffer (pH 7.4), 10 mM MgCl2, 25 μg ml−1 3-α-estradiol, UDPGA, and a selective substrate. The reactions were incubated with various substrates at 30 min at 37°C. The concentrations of estradiol, CDCA, 4-MU, MPA and naloxone were 20, 50, 100, 200 μM, respectively, all of which were the concentrations below apparent Km values. Other experimental conditions were the same as those described before. The enzymatic activity was determined by HPLC and LC-MS analysis of the produced metabolites of the respective substrates. Incubation conditions are listed in Supplementary Table 1.

**Quantification of bile acids in mice.** The abundance of bile acids across multiple tissue compartments was assayed. Bile acids were extracted in 70% ethanol at 55°C for 4 h, and bile salt species were analysed using a fast liquid chromatography-mass spectrometry (UFLC)-Triple-time of flight MS analysis. The alcohol extracts were subjected to further solid-phase extraction using solid-phase extraction technique (UCT-CLEAN UP C18, Oasis-HLB and Oasis-MAX cartridges), and then the bile acids were desalted using a ZOEBAX Eclipse Plus C18 column (150 × 2.1 mm, 3.5 μm) (Agilent Technologies, USA) protected by an SecurityGuard (Phenomenex Inc. CA, USA). The mobile phase (delivered at 0.2 ml min−1) consisted of (A) 2.6 mM mmol−1 ammonium acetate in water (adjusted to pH 6.8 with ammonium hydroxide) and (B) acetonitrile. The MS analysis was performed in a negative scan mode from m/z 50 to 1,000, and the expression pseudomolecular ion [M−H]− for each bile acid species was extracted for quantification under extracted ion chromatogram mode. The information of fragment ions produced at Q3 was used to ascertain the identity of bile acid species. The retention time, pseudomolecular ions and fragment ions for all the bile acids species are shown in Supplementary Table 6. Analyst 1.4.2 software (AB Sciex) was performed on AB SCIEX Triple TOF 5,600 mass spectrometer equipped with an atmospheric pressure chemical ionization interface. The chromatographic separation was achieved on an Agilent C18 column (150 × 2.1 mm, 3.5 μm) (Agilent Technologies, USA), protected by a SecurityGuard (Phenomenex Inc. CA, USA). The mobile phase comprising 0.1% formic acid and acetonitrile (B) was delivered at a flow rate of 0.25 ml min−1 using a gradient program set as: initial 30% B for 2 min, linear gradient 30–90% B from 3 to 5.5 min and to 95% B until 10 min, and then returned back to initial 30% in 2 min and maintained for a further 3 min for column balance. Selective ion monitoring was performed in the positive mode for the determination of pseudomolecular ions of OEA at m/z 326.2 and the IS propargonol at m/z 260.1. The amounts of OEA in tissues are expressed as micrograms per gram of wet tissue weight.

Statistical analysis. All experimental data are expressed as the mean ± s.e.m. as indicated in the figure legends. Differences among multiple groups were tested using one-way analysis of variance followed by Dunnett’s post-hoc comparisons. Differences between two groups were tested by the Student’s t-test. Differences were considered significant if P < 0.05 and have been marked with asterisks in the graph accordingly.

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**Author contributions**

H.H., X.Z., G.W. and F.J.G. designed the study; X.Z., L.C., C.J., H.H., Y.X., X.C., K.W.K., J.G., X.Z., L.C. and H.H. collected and analysed data; H.H., X.Z., G.W. and F.J.G. wrote the manuscript. X.Z., L.C. and C.J. contributed equally to this work.

**Additional information**

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