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Hepatocyte nuclear factor 4α in the intestinal epithelial cells protects against inflammatory bowel disease

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Abstract

Background—Hepatocyte nuclear factor 4α (HNF4α; NR2A1) is an orphan member of the nuclear receptor superfamily expressed in liver and intestine. While HNF4α expression is critical for liver function, its role in the gut and in the pathogenesis of inflammatory bowel disease (IBD) is unknown.

Methods—Human intestinal biopsies from control and IBD patients were examined for expression of mRNAs encoding HNF4α and other nuclear receptors. An intestine-specific HNF4α null mouse line (*Hnf4α*$^{\Delta IEpC}$) was generated using an *Hnf4α*-floxed allele and villin-Cre transgene. These mice and their control floxed counterparts (*Hnf4α*$^{F/F}$), were subjected to a dextran sulfate sodium (DSS)-induced IBD colitis protocol and their clinical symptoms and gene expression patterns determined.

Results—In human intestinal biopsies, HNF4α was significantly decreased in intestinal tissues from Crohn's disease and ulcerative colitis patients. HNF4α expression was also suppressed in the intestine of DSS-treated mice. In *Hnf4α*$^{\Delta IEpC}$ mice, disruption of HNF4α expression was observed in the epithelial cells throughout intestine. In the DSS-induced colitis model, *Hnf4α*$^{\Delta IEpC}$ mice showed markedly more severe changes in clinical symptoms and pathologies associated with IBD including loss of body weight, colon length, and histological morphology, as compared with *Hnf4α*$^{F/F}$ mice. Furthermore the *Hnf4α*$^{\Delta IEpC}$ mice demonstrate a significant alteration of mucin associated genes and increase intestinal permeability, which may play an important role in the increased susceptibility to acute colitis following an inflammatory insult.

Conclusions—While HNF4α does not have a major role in normal function of the intestine, it protects the gut against DSS-induced colitis.

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INTRODUCTION

Inflammatory bowel disease (IBD) is a group of inflammatory conditions of the large intestine and small intestine, including Crohn's disease (CD) and ulcerative colitis (UC) (1,2). The etiology of IBD is still not fully understood, however nuclear receptors have been shown to be critical in the pathogenesis of IBD; vitamin D receptor (VDR) (3,4), peroxisome proliferator-activated receptor (PPAR) γ (5–7), PPARα (8), PPARδ (9), pregnane X receptor (PXR) (10, 11), and glucocorticoid receptor (GR) (12,13) have been shown to influence the severity of IBD symptoms in experimental models. Interestingly, the relationship between the liver and gut-enriched nuclear receptor, hepatocyte nuclear factor 4α (HNF4α) and IBD has remained unexplored.

HNF4α, an orphan member of the nuclear receptor superfamily (NR2A1) (14,15), is expressed in liver, kidney, small intestine, colon, and also to some degree in pancreas (16,17). *Hnf4α*-null mice were shown to be embryonic lethal (18), indicating that HNF4α is an essential transcription factor during embryonic development. To assess the function of HNF4α gene in adult liver, liver-specific *Hnf4α*-null mice were established using the Cre-loxP method with albumin-Cre transgenic mice that specifically express the Cre gene in hepatocytes under the control of the rat albumin promoter (19). In liver, HNF4α was found to be critical in lipid, glucose, ammonia, and amino acid metabolism, and in the production of bile acids and blood coagulation via regulation of several liver-specific genes (19–24). Pancreatic β cell-specific *Hnf4α*-null mice were generated using rat insulin 2 gene promoter-regulated Cre transgenic mice (25). These mice exhibited impaired glucose-stimulated insulin secretion as well as human maturity onset diabetes of the young 1 (MODY1), but did not develop diabetes. However, another line of β cell-specific *Hnf4α*-null mice exhibited hyperinsulinemia and hypoglycemia (26). The discrepancies between two lines might be explained by differences in deleted exons flanked by loxP sites (exon 2, or exon 4 and 5, respectively) or genetic background.

Similar to hepatic HNF4α, HNF4α in the gut was reported to regulate the expression of many genes such as apolipoprotein A-I, A-IV, B, guanylyl cyclase C, CYP3A4, intestinal alkaline phosphatase, and meprin 1α in intestinal cells (27–33). Recently, embryo colon-specific *Hnf4α*-null mice were generated using Cre-loxP method with Foxa3-Cre transgenic mice (34). These mice revealed an essential role for HNF4α in development of the colon. However, the role of HNF4α in the adult gastrointestinal tract remains unknown.

In the current study, HNF4α expression was significantly decreased in IBD patients. To assess the function of HNF4α in adult intestine, an intestinal epithelial cell-specific *Hnf4α*-null mouse line was produced using Cre transgenic mice in which the Cre gene is expressed in the intestine under the control of the mouse villin promoter (35). The intestine-specific *Hnf4α*-null mice exhibited increased susceptibility to DSS-induced IBD including increased intestinal permeability. These findings suggest that HNF4α may have an important role in the etiology of IBD.

MATERIALS AND METHODS

Human IBD samples

To assess nuclear receptor gene expression in human IBD patient tissues, TissueScan Tissue qPCR Arrays Panels of human IBD patients were purchased from OriGene Technologies, INC. (Rockville, MD). The cDNAs per each panel were composed of 6 normal, 21 CD, and 21 UC patients (Table 1).

Generation of intestine-specific *Hnf4α*–null mice

Intestinal epithelial cells-specific *Hnf4α*-null mice, designated $Hnf4\alpha^{\Delta IEpC}$, were generated by crossing *Hnf4α*-floxed mice, designated $Hnf4\alpha^{F/F}$ (19) with mice carrying the villin-Cre transgene (35). Villin-cre transgenic mice were provided by Deborah L. Gumucio, University of Michigan. Cre-mediated recombination results in removal of exons 4 and 5 of the *Hnf4α* gene. The ($Hnf4\alpha^{flox/WT}$; Villin-Cre^{+}) F_1 mice were interbred with $Hnf4\alpha^{flox/flox}$ littermates lacking Villin-Cre. All mice were genotyped by PCR, and $Hnf4\alpha^{flox/flox}$; Villin-Cre^{+} (designated $Hnf4\alpha^{\Delta IEpC}$) and $Hnf4\alpha^{flox/flox}$; Villin-Cre^{-} ($Hnf4\alpha^{F/F}$) mice were used for the following experiments. PCR genotyping for *Hnf4α* floxed and recombined alleles, and the Cre/microsomal epoxide hydrolase (mEH) gene was carried out as described previously (19). All experiments were performed with 2-month-old $Hnf4\alpha^{F/F}$ and $Hnf4\alpha^{\Delta IEpC}$ mice. To assure genetic homogeneity between the $Hnf4\alpha^{F/F}$ and $Hnf4\alpha^{\Delta IEpC}$ mice, they were interbred for more than ten generations and littermates used in all experiments. Mice were housed in a pathogen-free animal facility under standard 12 hr light/12 hr dark cycle with ad libitum water and chow. All experiments with mice were carried out under Association for Assessment and Accreditation of Laboratory Animal Care guidelines with approval of the NCI Animal Care and Use Committee.

Northern blot analysis

Northern blot analysis was carried out as described previously (20). All probes were amplified from a mouse liver cDNA library using gene-specific primers and cloned into pCR II vector (Invitrogen, Carlsbad, CA). Sequences were verified using CEQ 2000 Dye Terminator cycle sequencing kit (Beckman Coulter, Fullerton, CA) with a CEQ 2000XL DNA Analysis System (Beckman Coulter).

Quantitative real-time PCR

Total RNA was isolated from each mouse tissues using Trizol reagent (Invitrogen) and was reverse-transcribed using random hexamers and Superscript II reverse transcriptase (Invitrogen). Quantitative real-time PCR (qPCR) was performed on an ABI PRISM 7900HT sequence detection system (Applied Biosystems, Foster City, CA) and PCR primers designed by Primer Express (Applied Biosystems). Purified cDNAs were amplified using SYBR Green PCR Master Mix (Applied Biosystems) and 0.3 mM specific oligonucleotide primers. The sequence and Genbank accession numbers for the primers used to quantify mRNAs in this study are available on request. PCR was performed at 95°C for 10 minutes followed by 40 cycles of 15 s at 95°C and 1 min at 60°C. Efficient amplification of each mRNA was confirmed using a cDNA dilution series. For each sample, the mean threshold cycle (Ct) from two replicate PCR using RNA isolated from independent colons was taken. Expression levels of mRNA were normalized to 36B4 RNA as internal standard by the comparative method. All values are expressed as the mean ± standard deviation.

Western blot analysis

Frozen colon tissues were gently homogenized in a glass tube with a manual pestle, and nuclear and cytoplasmic extracts were prepared using NE-PER nuclear and cytoplasmic extraction reagents (Pierce, Rockford, IL), with the addition of proteinase inhibitors (Roche inhibitor mixture set I and 1 mM phenylmethylsulfonyl fluoride). Nuclear or cytoplasmic protein (15–50 μg) was subjected to SDS-polyacrylamide gel electrophoresis (10–12.5%), followed by transfer to a polyvinylidene difluoride membrane (Amersham Biosciences, Piscataway, NJ). The membrane was incubated with phosphate-buffered saline containing 0.1% Tween 20 and 5% dry milk for 1 h and then overnight with a primary antibody against HNF4α (dilution 1:500) (Santa Cruz Biotechnology, Santa Cruz, CA) and β-actin (dilution 1:10,000) (Santa Cruz Biotechnology). After washing, the membrane was incubated with a 1:5,000 diluted

peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology), and the product was visualized using a chemiluminescent system (Super Signal West Pico Chemiluminescent Substrate; Pierce). The gels were scanned, and the bands were quantified by analysis of tagged image files using Image/J 1.36b software (Research Services Branch, National Institute of Mental Health, National Institutes of Health). The β-actin signals were used as loading controls for quantifying expression of HNF4α. The expression of HNF4α in HepG2 cell was used as positive control.

Histological and immunohistochemical analysis

Duodenum, jejunum, ileum, and colon from 2-month-old *Hnf4α*$^{F/F}$ and *Hnf4α*$^{\Delta IEpC}$ mice were fixed in 10% neutral buffered formalin and embedded in paraffin, and sections cut at a thickness of 3 μm were stained with hematoxylin and eosin (H&E), Alcian Blue (Sigma-Aldrich), and periodic acid-Schiff (PAS) (Sigma-Aldrich). Pieces of colon were fixed in 2.5% glutaraldehyde and postfixed in osmium tetroxide, and thin sections were stained with uranyl nitrate and lead citrate for the ultrastructural study. Immunohistochemical analysis was performed using antibody directed against HNF4α (Santa Cruz Biotechnology).

Induction and assessment of experimental DSS-induced IBD

To study the role of HNF4α in IBD, 2-month-old *Hnf4α*$^{F/F}$ and *Hnf4α*$^{\Delta IEpC}$ mice (n=14 for each group) were administered 2.5% DSS in the drinking water for five days. Daily changes in body weight and clinical signs of colitis, such as rectal bleeding, diarrhea, and bloody stool, were assessed and reported as a score from 0 to 4. At day 5 after DSS treatment, the mice were killed and colon tissues collected for histological study and mRNA gene expression. For histological analysis, colon tissue samples were H&E stained and histology score was analyzed (36). Other detailed experimental methods of DSS-induced IBD were described in previous reports (7,37).

In vivo intestinal permeability in DSS-induced IBD

Intestinal permeability was assayed in *Hnf4α*$^{F/F}$ and *Hnf4α*$^{\Delta IEpC}$ mice using a FITC-labeled-dextran method, as described previously (11). Untreated and three-day DSS treated mice were gavaged with 0.6 mg/g body weight of fluorescein isothiocyanate (FITC)–dextran solution (mol wt 4,000, at a concentration of 60 mg/ml; Sigma-Aldrich). Blood samples were collected from each group at 2 hr and 4 hr after FITC-dextran administration (n=4 for each group). A fluorescent unit of FITC-dextran in blood plasma was measured using fluorescent detection method.

Statistical analysis

All data are expressed as the mean ± SD. Statistical significance was determined by the Student *t* test for unpaired samples. For the analysis of human IBD patient data, Mann-Whitney U test was performed to assess differences between normal and patients group. P values are expressed as *, $P<0.05$, ** $P<0.01$, and ***, $P<0.001$.

RESULTS

Gene expression analysis for nuclear receptors in IBD patients

Gene expression analysis was performed for HNF4α, farnesoid X receptor (FXR), liver X receptor (LXR), mineralocorticoid receptor (MR), peroxisome proliferator-activated receptor α, β, γ (PPARα, PPARβ, PPARγ), vitamin D receptor (VDR), and pregnane X receptor (PXR) in intestinal samples from IBD patients (Fig. 1). The levels of mRNA encoding HNF4α, MR, PPARγ, and PXR was significantly decreased in intestinal samples from IBD patients compared to healthy controls, whereas no difference was observed in gene expression of LXR,

PPARα, PPARβ, and VDR. Interestingly, HNF4α, gene expression was significantly downregulated in both CD and UC patients suggesting HNF4α may play an important role in the pathogenesis of IBD.

HNF4α gene expression was decreased in experimental DSS-induced IBD

To investigate the role of HNF4α in experimental DSS-induced IBD, C57BL/6N mice were exposed to 2.5% or 5% (wt/vol) DSS for 7 days. The expression of HNF4α mRNA was markedly decreased, over 90% inhibition in experimental DSS-induced IBD samples (Fig. 2). Compared with untreated mice, both 2.5% DSS and 5% DSS treatment resulted in decreased HNF4α mRNA expression as revealed by quantitative real-time PCR (qPCR) (Fig. 2A). To investigate the time dependence on decreased HNF4α gene expression after DSS treatment, mice were killed and colon tissues were collected at day 1, 3, 5, and 7 following 2.5% DSS treatment. HNF4α mRNA expression was significantly decreased as early as day 1 following DSS adminstration (Fig. 2B). Consistent with these data, HNF4α protein levels were decreased (Fig. 2C) after 2.5% DSS treatment and the relative band density of HNF4α compared with β-actin was also decreased as early as day 1 after treatment (Fig. 2D). Therefore, these data suggest a role of HNF4α in an acute colon inflammatory model. To further investigate the role of HNF4α in colon inflammation, gut-specific conditional knockout mice were generated and examined.

Generation of intestinal epithelial cells-specific *Hnf4α*$^{\Delta IEpC}$ mice

Intestinal epithelial cell-specific *Hnf4α*-null mice were produced by crossing mice homozygous for *Hnf4α*-floxed alleles (*Hnf4α*$^{flox/flox}$) with villin-cre transgenic (Villin-Cre) mice (Fig 3A). The genotype was confirmed using PCR with primers specific for the *Hnf4α* floxed allele and Cre gene (Fig 3B). To assess the loss of HNF4α gene expression, northern blot analysis was performed using total RNA from 2-month-old *Hnf4α*$^{F/F}$ and *Hnf4α*$^{\Delta IEpC}$ mice (Fig. 3C). HNF4α mRNA in *Hnf4α*$^{F/F}$ mice was detected in all tissues examined, including liver, kidney, duodenum, jejunum, ileum, and colon (left panel, L, K, D, J, I, and C, respectively). In *Hnf4α*$^{\Delta IEpC}$ mice (right panel), loss of HNF4α mRNA was observed in duodenum, jejunum, ileum and colon, but not in liver and kidney. This result indicates that *Hnf4α*$^{\Delta IEpC}$ mice recombine the floxed allele specifically in intestinal epithelial cells. *Hnf4α*$^{\Delta IEpC}$ mice were viable and their growth rates normal when compared to *Hnf4α*$^{F/F}$ mice. By immunohistochemical analysis using HNF4α antibody, HNF4α protein was detected in the epithelial cells of duodenum, jejunum, ileum and colon of *Hnf4α*$^{F/F}$ mice (Fig. 3D; a, c, e, and Fig. 4C), but not in *Hnf4α*$^{\Delta IEpC}$ mice (Fig. 3D; b, d, f, and Fig. 4C), in agreement with results of mRNA expression.

Histological and immunohistochemical anlaysis of *Hnf4α*$^{\Delta IEpC}$ mice

To determine the effect of HNF4α disruption on intestinal epithelial cell morphology, histology was investigated (Fig. 4). H&E staining was produced in the duodenum (Fig. 4A; a and b), jejunum (Fig. 4A; c and d), ileum (Fig. 4A; e and f), and colon (Fig. 4A; g and h) between *Hnf4α*$^{F/F}$ and *Hnf4α*$^{\Delta IEpC}$ mice. In addition, since HNF4α was shown to be critical in embryonic development of the mouse colon (34), ultrastructure of the colon in *Hnf4α*$^{\Delta IEpC}$ mice was studied by electron microscopy. Colon epithelial cells in both *Hnf4α*$^{F/F}$ and *Hnf4α*$^{\Delta IEpC}$ mice were generally intact and no significant damages in sub-cellular organelles were noted. However, significant numbers of mucous granules were found in the epithelial cells of *Hnf4α*$^{\Delta IEpC}$ mice compared to *Hnf4α*$^{F/F}$ mice (Fig. 4B). In order to investigate biochemical differences in colon of *Hnf4α*$^{\Delta IEpC}$ mice, PAS and Alcian blue stain was performed for detection of polysaccharides including neutral mucopolysaccharides, and acidic mucopolysaccharides, respectively (Fig. 4C). The intensity of PAS staining in *Hnf4α*$^{\Delta IEpC}$ mouse gut was weaker than that in *Hnf4α*$^{F/F}$ mice (Fig. 4C; m and n); the same result was

obtained by Alcian blue staining (Fig. 4C; o and p), indicating that secretion of mucous from goblet cells may be reduced in the colon of *Hnf4α*$^{\Delta IEpC}$ mice.

Susceptibility of *Hnf4α*$^{\Delta IEpC}$ mice to experimental DSS-induced IBD

Hnf4α$^{\Delta IEpC}$ mice showed an increased susceptibility to DSS-induced IBD compared with *Hnf4α*$^{F/F}$ mice (n=14 for each group). Only 3 days after DSS treatment, *Hnf4α*$^{\Delta IEpC}$ mice showed significant differences in the extent of body weight loss compared with *Hnf4α*$^{F/F}$ mice (Fig. 5A). Body weight changes in *Hnf4α*$^{\Delta IEpC}$ and *Hnf4α*$^{F/F}$ mice were 83.1 ± 7.1 % and 98.1 ± 4.3 %, respectively at day 5 after DSS treatment relative to their initial weight (Fig. 5B). Colon length of *Hnf4α*$^{\Delta IEpC}$ mice with DSS treatment was considerably shortened compared with *Hnf4α*$^{F/F}$ mice and control mice without DSS treatment (Fig. 5, C and D). At day 5, the colon length of *Hnf4α*$^{\Delta IEpC}$ and *Hnf4α*$^{F/F}$ mice were 6.1 ± 1.1 cm and 8.5 ± 0.6 cm, respectively. *Hnf4α*$^{\Delta IEpC}$ mice showed more severe clinical symptoms such as diarrhea and bleeding compared to *Hnf4α*$^{F/F}$ mice (data not shown). Histological analysis of *Hnf4α*$^{\Delta IEpC}$ colonic tissue did not show significant morphological changes without DSS-treatment (Fig. 6, A and B). However, following 5-day DSS administration *Hnf4α*$^{\Delta IEpC}$ mice demonstrated absence of epithelium and intensive submucosal infiltration of inflammatory cells compared with DSS-treated *Hnf4α*$^{F/F}$ mice (Fig. 6, C and D). Histological score in *Hnf4α*$^{\Delta IEpC}$ mice was significantly higher compared with *Hnf4α*$^{F/F}$ mice following 5-day DSS treatment (Fig. 6E). These findings indicate that HNF4α in the intestinal epithelial cells has a protective role against DSS-induced IBD.

Analysis of cytokine expression levels in DSS-induced IBD

To analyze cytokine gene expression levels in *Hnf4α*$^{F/F}$ and *Hnf4α*$^{\Delta IEpC}$ mice with or without 5-day DSS treatment, qPCR was performed. IL-1β, IL-6, IL-10, TNFα, IFNγ, iNOS, CCR2, MCP-1, and ICAM-1 mRNA levels were elevated in colon tissues of *Hnf4α*$^{F/F}$ and *Hnf4α*$^{\Delta IEpC}$ mice after 5-day DSS treatment (Fig. 7). IL-6, IL-10, iNOS, MCP-1, and ICAM-1 mRNA expression showed no significant differences between *Hnf4α*$^{F/F}$ and *Hnf4α*$^{\Delta IEpC}$ mice. However, there were significant differences in IL-1β, TNFα, IFNγ, and CCR2 gene expression between *Hnf4α*$^{F/F}$ and *Hnf4α*$^{\Delta IEpC}$ mice after DSS treatment. Thus, lack of HNF4α in intestinal epithelium resulted in enhanced expression of IL-1β, TNFα, IFNγ, and CCR2 mRNA levels by DSS treatment. These data suggest that HNF4α in intestinal epithelium may have an important role in the regulation of chemokine signaling during insults that result in increased inflammation.

The expression of mucins, aquaporins, and meprins is altered in the colon of *Hnf4α*$^{\Delta IEpC}$ mice

Due to the decrease in mucous secretion in *Hnf4α*$^{\Delta IEpC}$ mice as revealed by electron microscopy and PAS and Alcian blue staining the expression of mucin (Muc), aquaporins (Aqp), and meprins (Mep) mRNAs were measured by qPCR. Loss of HNF4α mRNA in colon from *Hnf4α*$^{\Delta IEpC}$ mice was confirmed by qPCR (Table 2). As a positive control, the expression of meprin 1α (*Mep1α*), a direct target gene of HNF4α (33) and a metalloproteinase (38), was markedly decreased in *Hnf4α*$^{\Delta IEpC}$ mice. Similarly, decreased expression of *Mep1β*, a gene related to *Mep1α*, was observed in *Hnf4α*$^{\Delta IEpC}$ mice. Since the colon plays a major role in absorption of water, the expression of aquaporins (Aqp) that are involved in permeability of water and small solutes (39,40) was also investigated. The mRNA expression of Aqp1 and Aqp8 was markedly reduced, and that of Aqp4 was slightly increased in *Hnf4α*$^{\Delta IEpC}$ mice. Mucin is involved in disorders of the gastrointestinal tract and colorectal cancer (41,42). The expression of Muc3 was markedly decreased while Muc1 was increased, and Muc4, Muc5AC, Muc5B, and Muc6 were slightly increased in *Hnf4α*$^{\Delta IEpC}$ mice.

In vivo intestinal permeability in DSS-induced IBD

Mucin molecules have been shown to be critical in gastrointestinal barrier function; mucin molecules prevent bacterial colonization and increase bacterial clearance and serve to neutralize the acidic environment of the luminal colon. Due to the severe alteration in mucin gene expression and mucous secretions, intestinal permeability was assessed in *Hnf4α*$^{F/F}$ and *Hnf4α*$^{\Delta IEpC}$ mice (Fig. 8). Using a FITC-labeled-dextran method, there was no significant difference in intestinal permeability between *Hnf4α*$^{F/F}$ and *Hnf4α*$^{\Delta IEpC}$ mice without DSS-treatment. However, upon DSS treatment, *Hnf4α*$^{\Delta IEpC}$ mice showed a significantly higher permeability compared with *Hnf4α*$^{F/F}$ mice and both *Hnf4α*$^{F/F}$ and *Hnf4α*$^{\Delta IEpC}$ mice after 3-day DSS treatment showed a significantly higher permeability compared with untreated mice at 2, 4, and 8 hr after FITC-dextran administration. These results suggest that *Hnf4α*$^{\Delta IEpC}$ mice may be more susceptible to colon injury due to increase in intestinal permeability following an inflammatory insult.

DISCUSSION

Recently, it was reported that several nuclear receptors have a crucial role in the pathogenesis of IBD. In the present study intestinal samples from IBD patients demonstrated a significant decrease in the levels of mRNAs encoding HNF4α, MR, PPARγ, and PXR. The decrease in MR, PPARγ, and PXR is consistent with earlier studies (5,7,10,11,43,44). However, the relationship between HNF4α and IBD has not been reported. HNF4α is abundantly expressed in small intestine and colon. The HNF4α protein is also expressed in the nucleus of mucosal epithelial cells of intestine and colon (17), but the function of intestinal HNF4α is not known in contrast to hepatic HNF4α. Therefore, intestinal epithelial cell-specific *Hnf4α*-null mice, *Hnf4α*$^{\Delta IEpC}$, were generated and the loss of HNF4α mRNA and protein was clearly observed in the small intestine and colon.

To examine the role of HNF4α in IBD, a DSS-induced IBD model was performed with *Hnf4α*$^{F/F}$ and *Hnf4α*$^{\Delta IEpC}$ mice. As an experimental animal model, administration of dextran sulfate sodium (DSS) in the drinking water is widely used to induce IBD (36,45). DSS is a sulfated polymer that induces mucosal injury and results in inflammatory bowel disease-like colitis. The first insult occurring in the colonic mucosa is known to be the breakdown of the epithelial mucosal barrier and this breakdown of the intestinal barrier defense is thought to be important in pathogenesis of IBD (46,47). When mice were exposed to DSS treatment for 7 days, HNF4α expression was markedly decreased at both the mRNA and protein levels similar to human IBD patients. In agreement with a critical role for HNF4α in IBD, *Hnf4α*$^{\Delta IEpC}$ mice demonstrated an increased susceptibility to DSS-induced colitis compared with *Hnf4α*$^{F/F}$ mice as assessed by body weight, colon length, and histological analysis. In addition, prolonged treatment with DSS decreased the survival of *Hnf4α*$^{\Delta IEpC}$, whereas no change was observed in *Hnf4α*$^{F/F}$ mice (data not shown). In the mucosa of patients with IBD, several proinflammatory and immune-regulatory cytokines are upregulated (48). The increased levels of IL-1β and TNFα, as one of major proinflammatory cytokines, were detected in mucosal specimens from patients with IBD (49–51). Consistent with the increase in susceptibility to IBD, several cytokines were significantly increased in *Hnf4α*$^{\Delta IEpC}$ mice following DSS treatment compared to DSS-treated *Hnf4α*$^{F/F}$ mice (IL-1β, TNFα, IFNγ, and CCR2). Recently, a crosstalk between HNF4α and nuclear factor κB (NF-κB), a major regulatory gene in the inflammatory signaling pathway, was reported (52–54). It is possible that NF-κB-mediated crosstalk with HNF4α in intestine might be related to increase in cytokine expression in the absence of Hnf4α, but the mechanistic link is still unclear.

Also, altered expression of several mucins and aquaporins were found. Relative mRNA expression of Muc3, Aqp1, Aqp8, and Mep1α and 1β in the colon of *Hnf4α*$^{\Delta IEpC}$ mice was markedly decreased. Other genes, such as Muc4, Muc5AC, Muc5B, Muc6, and Aqp4, were

slightly induced less than two-fold in *Hnf4α*$^{\Delta IEpC}$ mice. Mucins are divided into two major classes by their structure and function; membrane-bound and secreted gel-forming mucins, respectively (41,42,55). Muc3 is a membrane-bound protein and the expression is also decreased in embryonic colon-specific *Hnf4α*-null mice. In agreement with this result, HNF4α binds to the upstream promoter region of the Muc3 gene *in vivo* (34). Several mucin genes including Muc3 are clustered at human chromosome 7q22 which was predicted to be a susceptibility locus for inflammatory bowel disease (IBD) (56). In addition, an association of rare alleles of the Muc3 gene and ulcerative colitis, a common form of IBD, was also suggested (57), indicating that HNF4α might be partly involved in IBD by regulating the expression of Muc3. Muc1 is a membrane-bound mucin (41,42,55) and its expression was strongly increased in the colon of *Hnf4α*$^{\Delta IEpC}$ mice. The expression of Muc1 is very low in normal intestinal epithelial cells, but increased in colorectal carcinoma (58,59). The cytoplasmic domain of Muc1 has the potential to associate with β-catenin (60,61) and thus, overexpression of Muc1 in the colon of *Hnf4α*$^{\Delta IEpC}$ mice might have a role in the development of colon cancer. Recently, it was reported that IL-10-deficent mice, a widely accepted mouse model of IBD, crossed to Muc1 overexpressing mice, accelerated the pathogenesis of IBD (62). It was previously demonstrated that MUC1 can acts as a chemotactic agent capable of recruiting inflammatory cells. In addition lectin receptors on macrophages are capable of binding to MUC1 and activating the innate immune cells. Therefore, the dysregulation of mucin expression may have a profound effect in the innate immune system, thus increasing the susceptibility of the *Hnf4α*$^{\Delta IEpC}$ mice to inflammatory insults (63–65).

Aqp8 is expressed gastrointestinal tract including colon (66,67) and *Aqp8*-null mice only exhibited a minor phenotype (68). However, expression of Aqp8 was decreased in a DSS-treated mice (69) and colorectal tumors (70). Similar to Aqp8, Aqp1 is also expressed in the gastrointestinal tract (66). Of interest, *Aqp1*-null mice were 10–15% smaller than wild-type mice (66). Since body weight of *Hnf4α*$^{\Delta IEpC}$ mice was unchanged as compared to *Hnf4α*$^{F/F}$ mice (data not shown), decreased expression of Aqp1 in the colon might not be critical for the loss of body weight. However, the role of Aqp1 in colon has not been elucidated. Meprins are metalloproteinases that are highly expressed in the intestinal epithelial cells and composed of two related subunits, α and β, leading to homo- or hetero-oligometric proteins (38). These enzymes degrade a wide range of proteins of extracellular matrix and biologically active peptides (71,72) and have been shown to be involved in cancer and intestinal inflammation (38,73,74). Since the expression of Mep1α is known to be dependent on HNF4α expression and an HNF4α binding site in the promoter region (33), Mep1α could be a direct target for HNF4α in the colon and thus may be of value to determine whether HNF4α is involved in Mep1β activation. Furthermore, since *Mep1β*-null mice only exhibited decreased levels of Mep1α in the intestine (75), the physiological role of decreased expression of Mep1α and 1β in the colon remains unknown. However, altered expression of other mucins and aquaporins may suggest a relationship with IBD.

Another possible molecular mechanism by which HNF4α influences IBD is through the regulation of intestinal permeability. *Hnf4α*$^{\Delta IEpC}$ mice showed a markedly higher intestinal permeability compared with *Hnf4α*$^{F/F}$ mice after DSS treatment. This increased intestinal permeability in *Hnf4α*$^{\Delta IEpC}$ mice after DSS treatment is consistent with increased histological inflammatory status and altered gene expression of mucins and aquaporins. During intestinal inflammatory insults, improvement in mucosal integrity related with HNF4α may reduce intestinal inflammation and protect against endotoxins or xenobiotics like DSS in intestinal tissue.

In conclusion, mice lacking expression of the HNF4α gene in intestinal epithelial cells were successfully developed. These mice exhibited decreased levels of polysaccharides and acidic mucopolysaccharides and altered expression of several genes encoding mucins and aquaporins.

Gut-specific *Hnf4α*-null mice showed higher susceptibility to DSS-induced IBD as a result of increased permeability of the epithelial barrier. Thus, HNF4α may have an important protective role in inflammatory bowel disease.

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Abbreviations

HNF4α
: hepatocyte nuclear factor 4α

IBD
: inflammatory bowel disease

CD
: Crohn's disease

UC
: ulcerative colitis

DSS
: dextran sulfate sodium

IEpC
: intestinal epithelial cell

F
: floxed allele

MODY1
: maturity onset diabetes of the young 1

H&E
: hematoxylin and eosin

PAS
: periodic acid-Schiff

Muc
: mucin

Aqp
: aquaporin

Mep
: meprin

FITC
: fluorescein isothiocyanate

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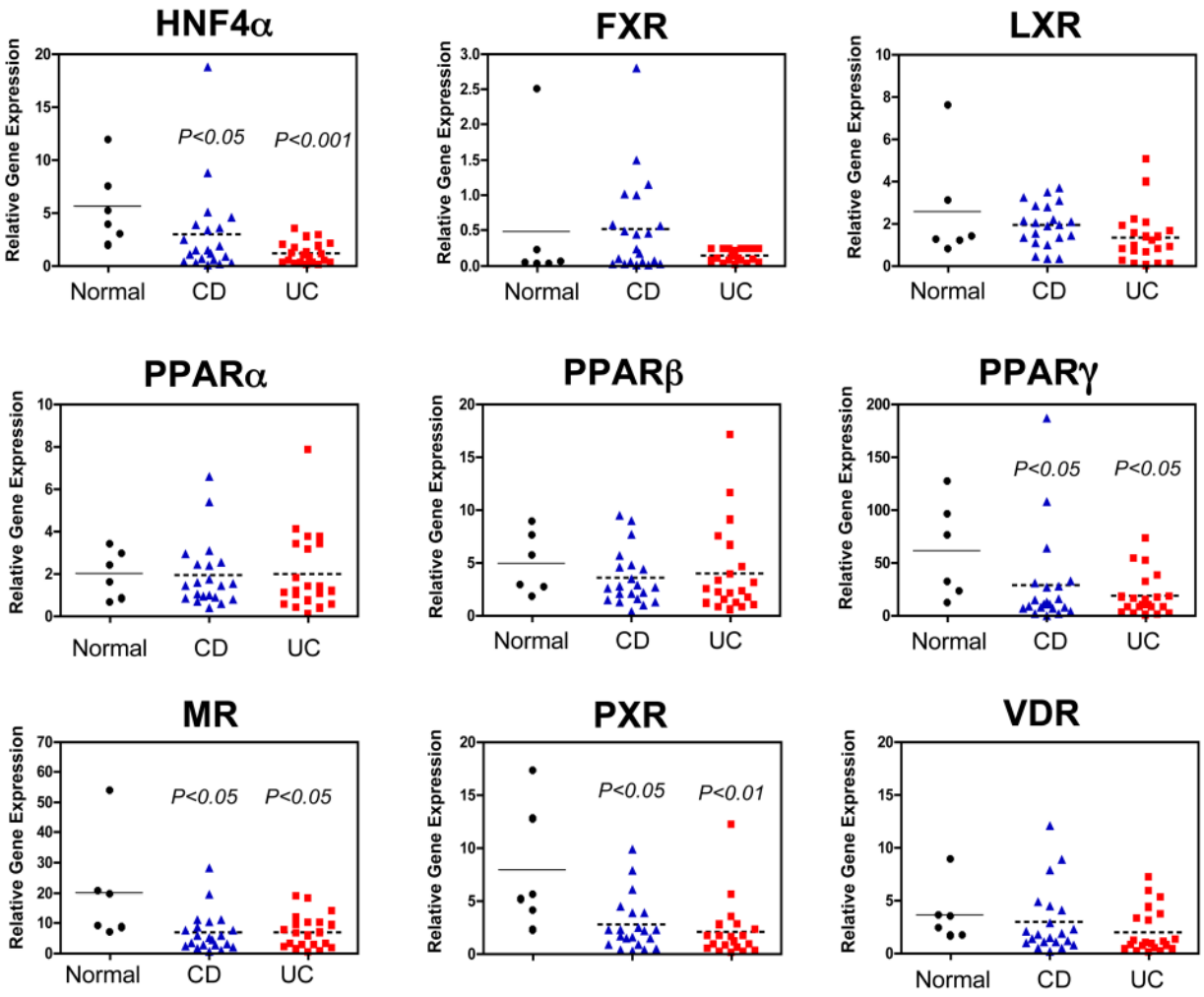

Fig. 1.

Nuclear receptor gene expression in intestinal samples from human IBD patients. The gene expression of hepatocyte nuclear factor 4α (HNF4α), farnesoid X receptor (FXR), liver X receptor (LXR), mineralocorticoid receptor (MR), peroxisome proliferator-activated receptor α, β, γ (PPARα, PPARβ, PPARγ), vitamin D receptor (VDR), and pregnane X receptor (PXR) were performed by qPCR to assess their gene expression in human IBD patient tissues. Mann-Whitney U test was performed to assess significant differences between normal and patients group. P values are expressed as *, *P<0.05*, ** *P<0.01*, and ***, *P<0.001*.

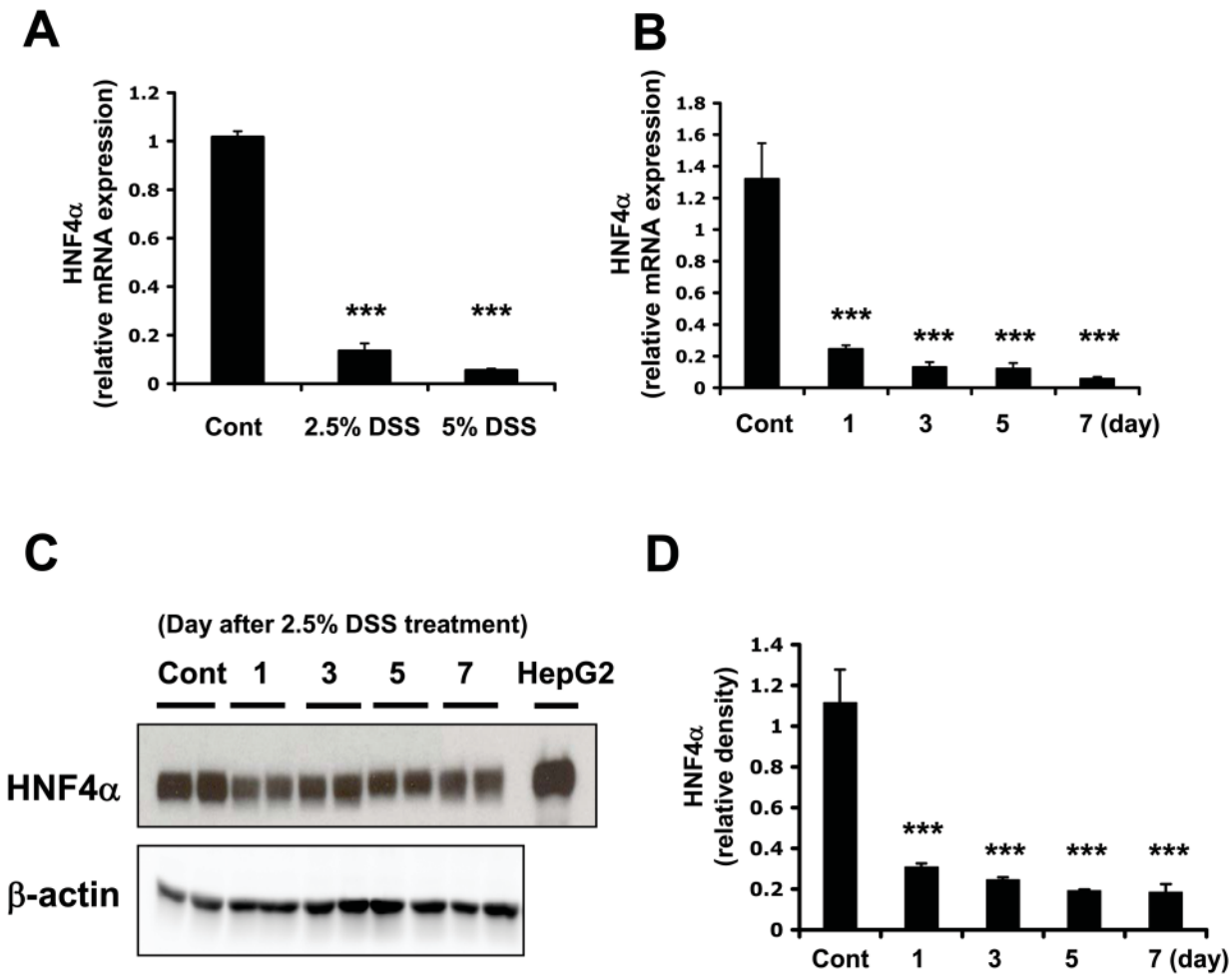

Fig. 2.

HNF4α gene expression after DSS treatment. Expression of HNF4α mRNA, as measured by quantitative real-time PCR (qPCR), was decreased at day 7 after 2.5% DSS and 5% DSS treatment (A). Expression of HNF4α mRNA was decreased at day 1, 3, 5, and 7 after 2.5% DSS treatment (B). HNF4α protein, as measured by western blot analysis, was also decreased at day 1, 3, 5, and 7 after 2.5% DSS treatment and relative density to β-actin was decreased at day 1, 3, 5, and 7 after 2.5% DSS treatment (C). Both relative mRNA and protein levels of HNF4α were decreased after DSS treatment. Data represent the mean value ± standard deviations, ***, $p<0.001$ compared with control mice.

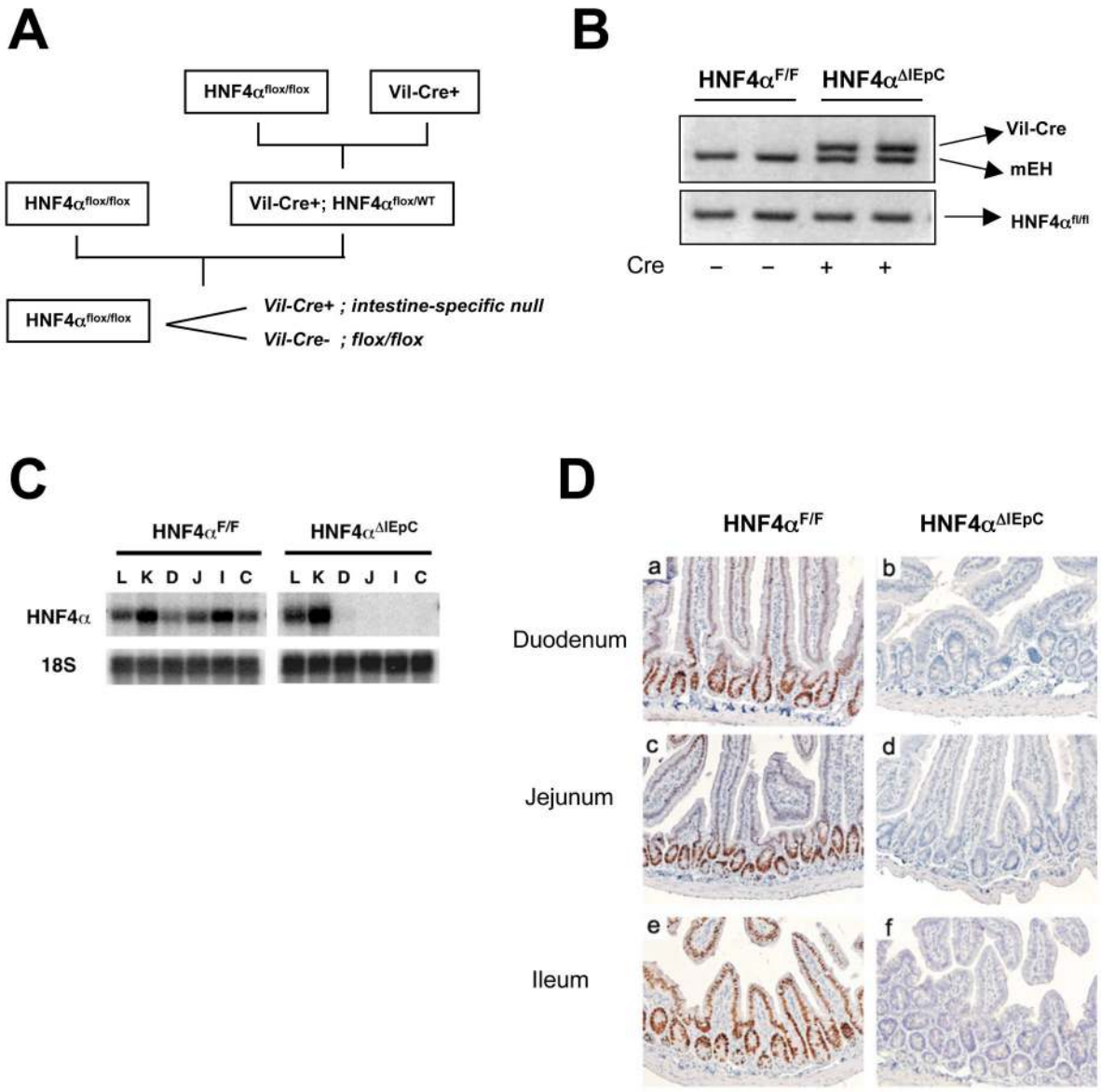

Fig. 3.

Generation of the intestinal epithelial cells-specific *Hnf4α*-null, *Hnf4α*$^{\Delta IEpC}$ mice. Schematic of generation of intestinal specific *Hnf4α*-null mice (A) and their genotypes by PCR (B) using microsomal epoxide hydrolase (mEH) primers as a positive control for amplication. The loss of intestine-specific HNF4α gene were analyzed by northern blot analysis (C) and immunohistochemical analysis (D). Immunohistochemistry analysis with HNF4α antibody of *Hnf4α*$^{F/F}$ (D; a, c, and e) and *Hnf4α*$^{\Delta IEpC}$ (D; b, d, and f) mice in duodenum (a and b), jejunum (c and d), ileum (e and f). Note the loss of HNF4α-positive cells in nucleus from *Hnf4α*$^{F/F}$ mice. HNF4α related gene expression as assessed by northern blot analysis in whole intestine of *Hnf4α*$^{F/F}$ and *Hnf4α*$^{\Delta IEpC}$ mice. Pooled total RNA (*n*=7 for each genotype) was isolated from liver, kidney, duodenum, jejunum, ileum, and colon (L, K, D, J, I, and C, respectively).

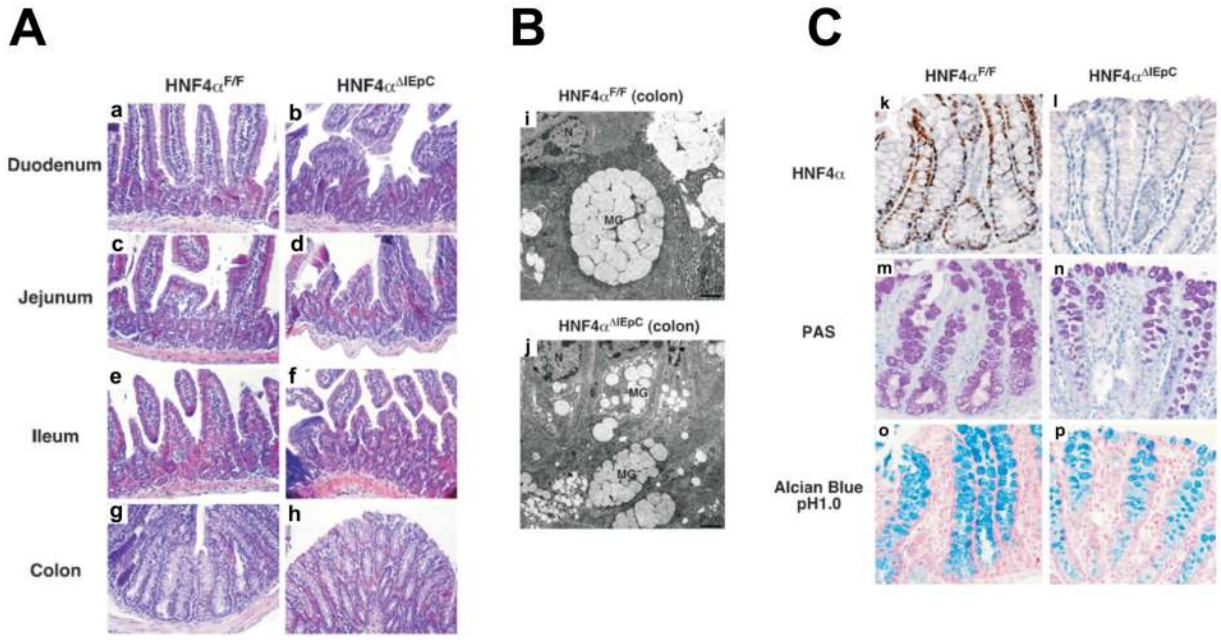

Fig. 4.

Histological and immunohistochemical analysis of the intestine in *Hnf4α*$^{F/F}$ and *Hnf4α*$^{\Delta IEpC}$ mice by H&E stained intestine sections (A), ultrastructure (B), PAS, and alcian blue stain(C). H&E stained intestine sections from *Hnf4α*$^{F/F}$ (A; left panel; a, c, e, and g) and *Hnf4α*$^{\Delta IEpC}$ (A; right panel; b, d, f, and h) mice (original magnification X 200) and ultrastructure of the colon from *Hnf4α*$^{F/F}$ (i) and *Hnf4α*$^{\Delta IEpC}$ (j) mice. N; nucleus, MG; mucous granules. Immunohistochemistry of HNF4α, and polysaccharide and acidic mucopolysaccharide stain in the colon of *Hnf4α*$^{F/F}$ and *Hnf4α*$^{\Delta IEpC}$ mice (C). Immunohistochemistry against HNF4α antibody (k and l), PAS (m and n), and Alcian blue stain (o and p) from in the colon of *Hnf4α*$^{F/F}$ (C; left panel) and *Hnf4α*$^{\Delta IEpC}$ (C; right panel) mice.

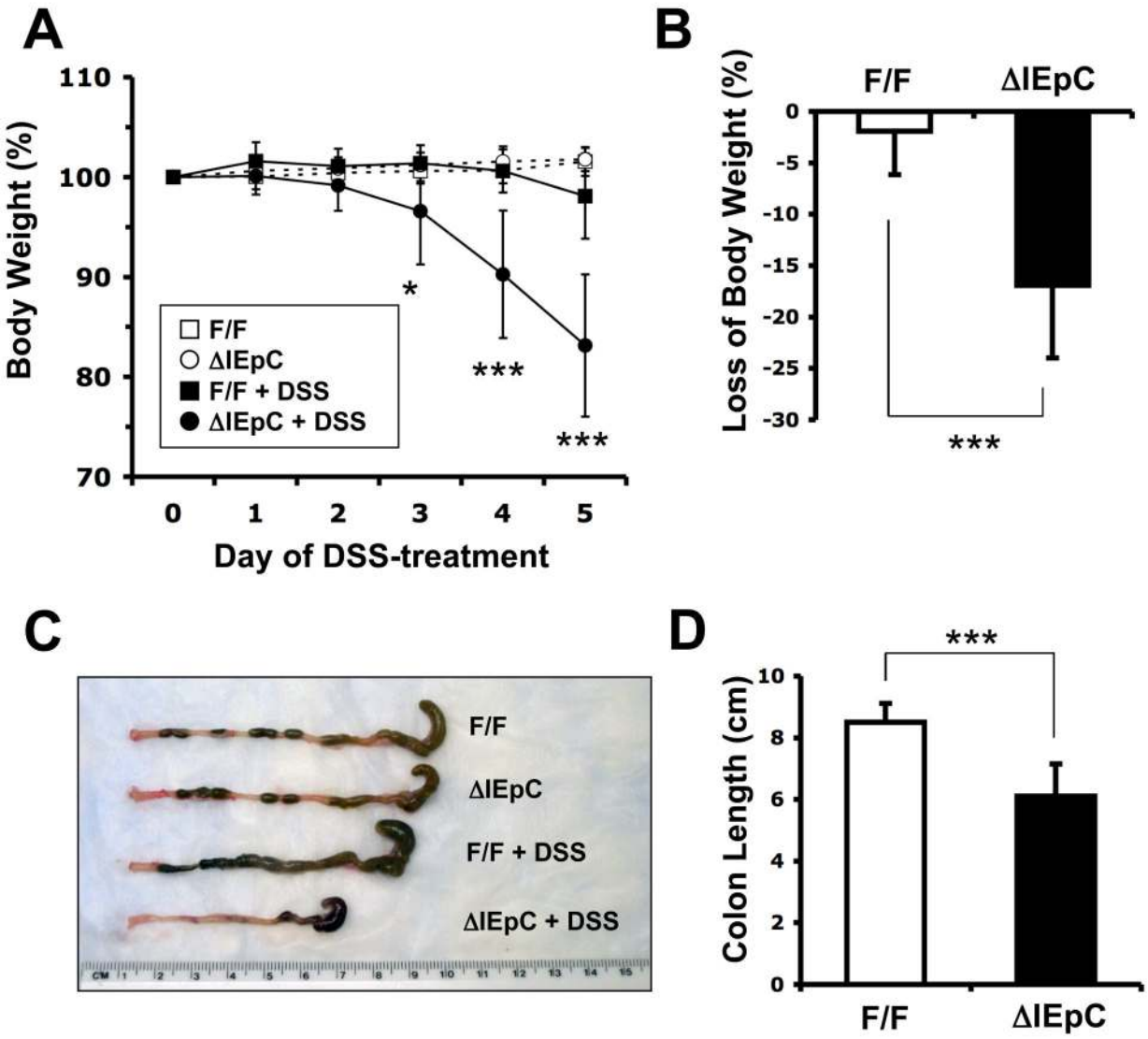

Fig. 5.

Assessment of DSS-induced IBD in *Hnf4α*$^{F/F}$ and *Hnf4α*$^{\Delta IEpC}$ mice. Lack of HNF4α in intestinal epithelial cells is associated with increased susceptibility to DSS-induced IBD. Daily changes in body weight following DSS treatment (A), loss of body weight at day 5 after DSS treatment (B), picture of the isolated colon from *Hnf4α*$^{F/F}$ and *Hnf4α*$^{\Delta IEpC}$ mice with or without DSS treatment (C), and colon length, which was shortened by severe IBD (D). Data represent the mean value ± standard deviations of n=14 for each group, *, $p<0.05$, and ***, $p<0.001$ compared with DSS-treated *Hnf4α*$^{F/F}$ mice.

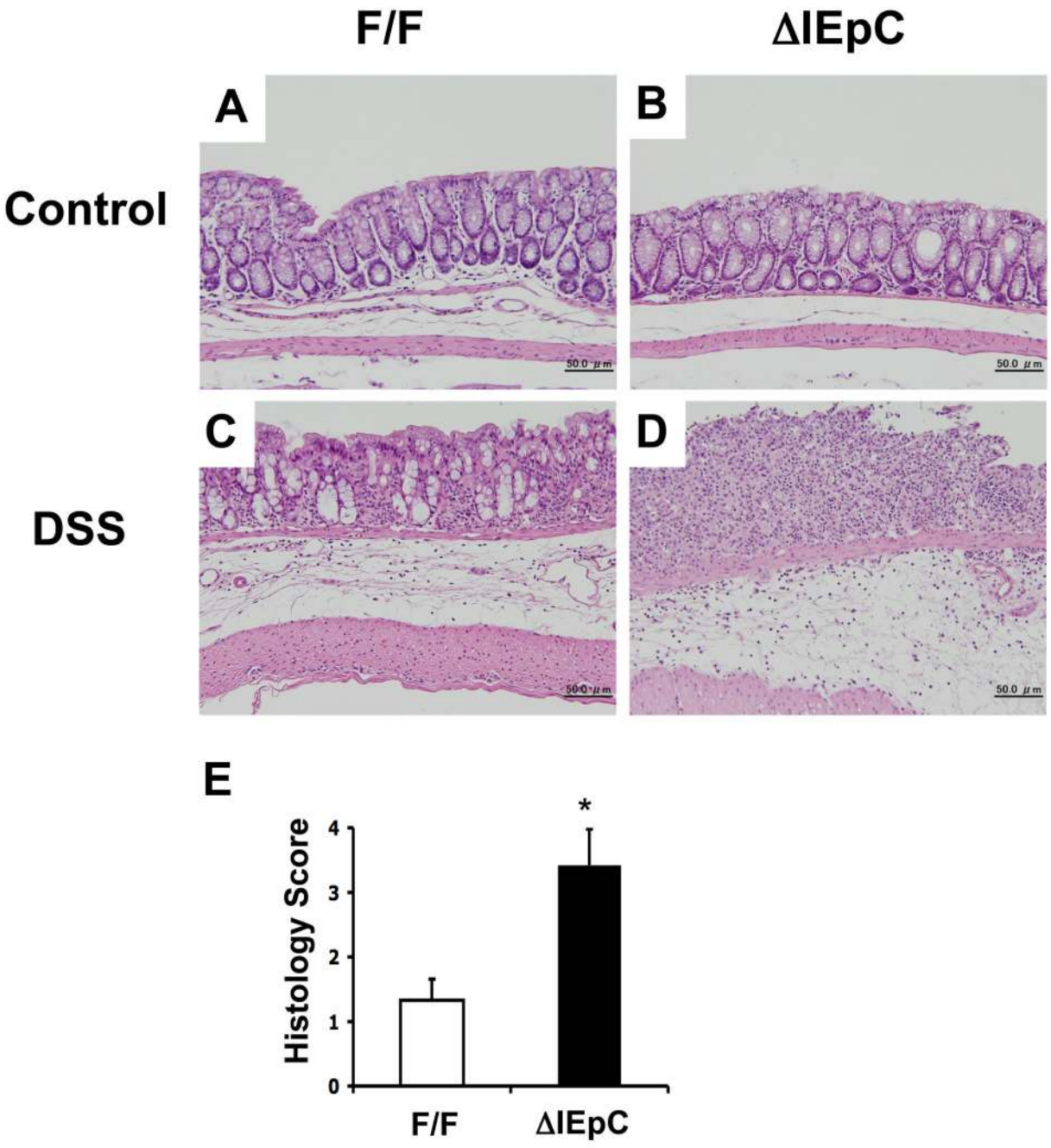

Fig. 6.

Histological assessment of 5-day DSS or control treatment in *Hnf4α*$^{F/F}$ and *Hnf4α*$^{\Delta IEpC}$ mice. H&E stained colon sections from *Hnf4α*$^{F/F}$ (A) and *Hnf4α*$^{\Delta IEpC}$ (B) mice following control treatment, *Hnf4α*$^{F/F}$ (C) and *Hnf4α*$^{\Delta IEpC}$ (D) mice after 5-day DSS treatment. Histological score was analyzed from colonic H&E stained sections of *Hnf4α*$^{F/F}$ and *Hnf4α*$^{\Delta IEpC}$ mice after 5-day DSS treatment (E). Data represent the mean value ± standard deviations, *, $p<0.05$ compared with DSS-treated *Hnf4α*$^{F/F}$ mice.

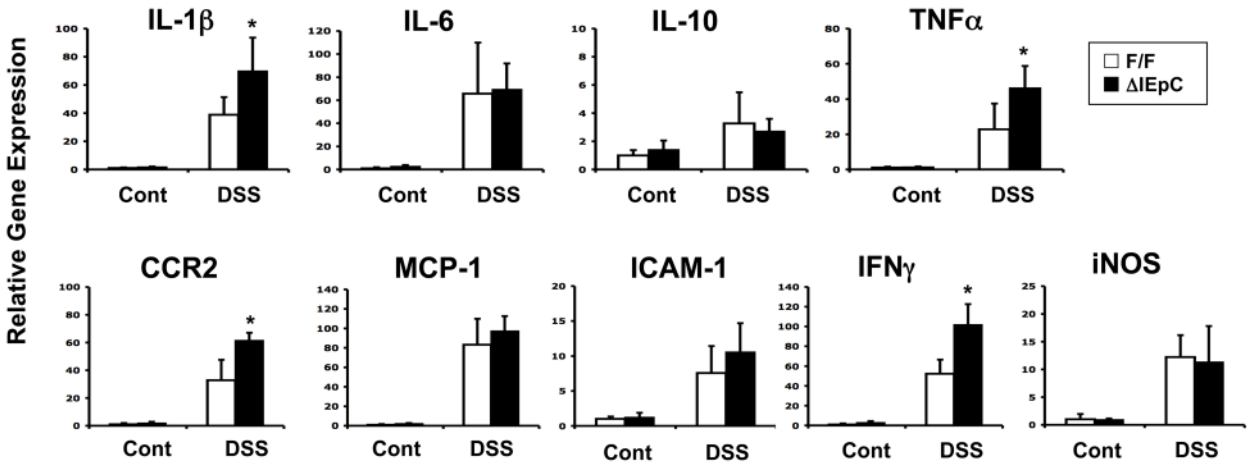

Fig. 7.

Cytokine gene expression from colon tissue following 5-day DSS or control treatment in *Hnf4α*$^{F/F}$ and *Hnf4α*$^{\Delta IEpC}$ mice. IL-1β, IL-6, IL-10, TNFα, IFNγ, iNOS, CCR2, MCP-1, and ICAM-1 mRNA expressions were analyzed by qPCR. Expression was normalized to 36B4. Data represent the mean value ± standard deviations of n=8 for each group, *, $p<0.05$ compared with DSS-treated *Hnf4α*$^{F/F}$ mice.

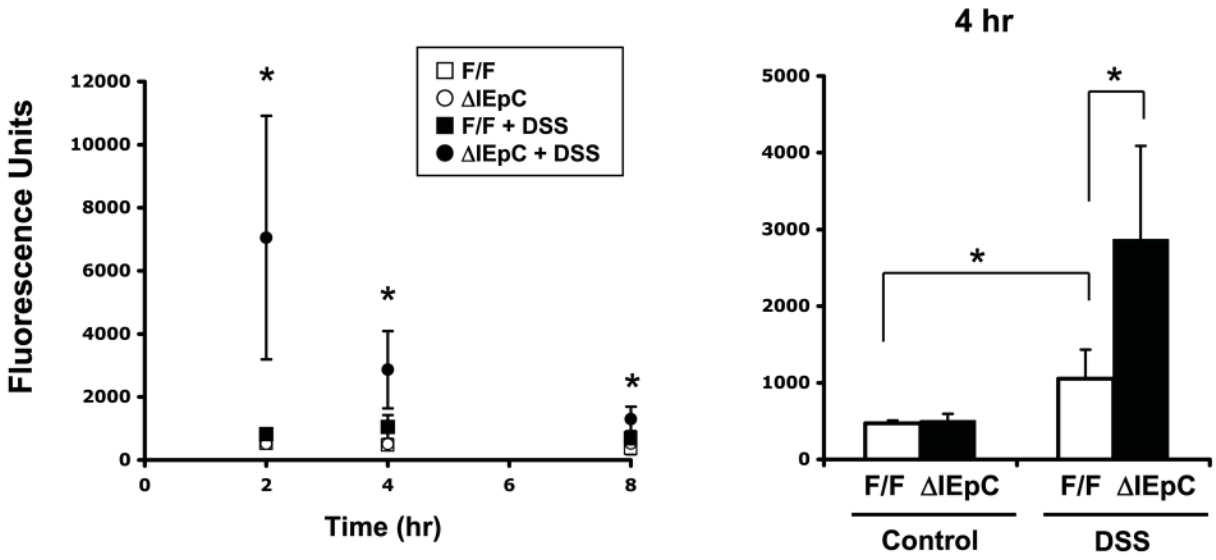

Fig. 8.

In vivo intestinal permeability assay. Intestinal permeability using a FITC-labeled-dextran method were measured at 2, 4, and 8 hr of fluorescence units after FITC-dextran oral administration following 3-day DSS treatment between *Hnf4α*$^{F/F}$ and *Hnf4α*$^{\Delta IEpC}$ mice. Data represent the mean value ± standard deviations, * $p<0.05$.

Table 1

Human IBD patient description

Patient #	Sex	Age	Diagnosis	Tissue	Patient #	Sex	Age	Diagnosis	Tissue
1	Male	78	Normal	Colon	25	Male	29	CD	Ileum
2	Male	47	Normal	Colon	26	Male	42	CD	Sm Intest
3	Female	31	Normal	Colon	27	Female	39	CD	Ileum
4	Male	54	Normal	Colon	28	Male	54	UC	Colon
5	Male	37	Normal	Ileum	29	Male	59	UC	Colon
6	Female	61	Normal	Ileum	30	Male	72	UC	Colon
7	Male	56	CD	Colon	31	Male	41	UC	Colon
8	Male	33	CD	Colon	32	Female	54	UC	Rectum
9	Female	46	CD	Colon	33	Female	72	UC	Colon
10	Female	51	CD	Colon	34	Female	36	UC	Colon
11	Female	31	CD	Ileum	35	Male	56	UC	Colon
12	Male	37	CD	Ileum	36	Male	31	UC	Rectum
13	Male	48	CD	Ileum	37	Male	23	UC	Colon
14	Female	65	CD	Ileum	38	Male	42	UC	Colon
15	Female	26	CD	Ileum	39	Male	42	UC	Colon
16	Male	33	CD	Cecum	40	Male	68	UC	Rectum
17	Female	45	CD	Sm Intest	41	Male	45	UC	Rectum
18	Male	64	CD	Ileum	42	Female	60	UC	Colon
19	Female	31	CD	Ileum	43	Male	33	UC	Colon
20	Male	35	CD	Colon	44	Female	27	UC	Colon
21	Male	20	CD	Ileum	45	Male	57	UC	Rectum
22	Male	41	CD	Colon	46	Female	35	UC	Colon
23	Female	21	CD	Colon	47	Female	30	UC	Colon
24	Male	41	CD	Colon	48	Female	26	UC	Colon

NOTE. CD; Crohn's disease, UC; ulcerative colitis, Sm Intest; small intestine.

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Table 2

Gene expression analysis in the colon of *Hnf4α*$^{F/F}$ and *Hnf4α*$^{\Delta IEpC}$ mice

Gene	*Hnf4α*$^{F/F}$	*Hnf4α*$^{\Delta IEpC}$
HNF4α	1.00 ± 0.16	0.01 ± 0.00***
Muc1	1.00 ± 0.34	7.54 ± 1.42***
Muc2	1.00 ± 0.49	0.88 ± 0.41
Muc3	1.00 ± 0.38	0.07 ± 0.05***
Muc4	1.00 ± 0.34	1.64 ± 0.47*
Muc5AC	1.00 ± 0.39	1.74 ± 0.46*
Muc5B	1.00 ± 0.35	1.77 ± 0.51*
Muc6	1.00 ± 0.36	1.54 ± 0.39*
Aqp1	1.00 ± 0.43	0.32 ± 0.10**
Aqp4	1.00 ± 0.33	1.50 ± 0.39*
Aqp8	1.00 ± 0.21	0.15 ± 0.03***
Mep1α	1.00 ± 0.30	0.12 ± 0.05***
Mep1β	1.00 ± 0.32	0.10 ± 0.04***

NOTE. Quantitative real-time PCR of colon mRNA from 2-month-old mice (n=6). All the values are means ± SD. Significant difference compared to *Hnf4α*$^{F/F}$ mice:

*, $p < 0.05$

**, $p < 0.01$

***, $p < 0.001$.