Tumor necrosis factor α upregulates the bile acid efflux transporter OATP3A1 via multiple signaling pathways in cholestasis

Revised as a Research Article

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Cholestasis is a common condition in which the flow of bile from the liver to the intestines is inhibited. It has been shown that organic anion–transporting polypeptide 3A1 (OATP3A1) is upregulated in cholestasis to promote bile acid efflux transport. We have previously shown that the growth factor fibroblast growth factor 19 and inflammatory mediator tumor necrosis factor α (TNFα) increased OATP3A1 mRNA levels in hepatoma peritoneal lavage cell/PRF/5 cell lines. However, the mechanism underlying TNFα-stimulated OATP3A1 expression in cholestasis is unknown. To address this, we collected plasma samples from control and obstructive cholestasis patients and used ELISA to detect TNFα levels. We found that the TNFα levels of plasma and hepatic mRNA transcripts were significantly increased in obstructive cholestatic patients relative to control patients. A significant positive correlation was also observed between plasma TNFαs and liver OATP3A1 mRNA transcripts in patients with obstructive cholestasis. Further mechanism analysis revealed that recombinant TNFα induced OATP3A1 expression and activated NF-κB and extracellular signal–regulated kinase (ERK) signaling pathways as well as expression of related transcription factors p65 and specificity protein 1 (SP1). Dual-luciferase reporter and chromatin immunoprecipitation assays showed that recombinant TNFα upregulated the binding activities of NF-κB p65 and SP1 to the OATP3A1 promoter in peritoneal lavage cell/PRF/5 cells. These effects were diminished following the application of NF-κB and ERK inhibitors BAY11-7082 and PD98059. We conclude that TNFα stimulates hepatic OATP3A1 expression in human obstructive cholestasis by activating NF-κB p65 and ERK–SP1 signaling. These results suggest that TNFα-activated NF-κB p65 and ERK–SP1 signaling may be a potential target to ameliorate cholestasis-associated liver injury.

Cholestasis is characterized by large amounts of bile acid (BA) accumulation in hepatocytes and serum (1). During an adaptive response to cholestasis, liver injury was attenuated because of the repression of BA synthesis via downregulation of CYP7A1 and CYP8B1 genes, and the enhancement of BA efflux via upregulation of multidrug resistance–associated protein 4, multidrug resistance–associated protein 3 (MRP3), organic anion–transporting polypeptide 3A1 (OATP3A1) protein, bile salt export pump, and organic solute transporter α/β (1–5). These BA transporters played crucial roles in maintaining BA homeostasis. Interestingly, emerging evidence showed that inflammation, as another part of adaptive response for cholestasis, was involved in regulating the expression of BA transporters and liver injury (2, 6). However, the mechanisms how inflammatory factors regulate adaptive expression of BA transporters is unclear.

OATP3A1 (known as SLC39A1) is expressed in multiple tissues (6–12) including the liver and functions as a membrane transporter mediating estrone-3-sulfate, prostaglandins E1 and E2, and thyroxine uptake (12–15). Previously, we have shown that OATP3A1 regulates the transport of BA efflux in cholestasis (6). OATP3A1 knockdown in mice aggravated intrahepatic BA accumulation and cholestatic liver injury (6), indicating that it has a critical role in cholestasis. Moreover, elevation of serum fibroblast growth factor 19 (FGF19) and tumor necrosis factor α (TNFα) has been detected in human obstructive cholestasis (16). We have previously shown that recombinant FGF19 and TNFα stimulated OATP3A1 expression in hepatoma peritoneal lavage cell (PLC)/PRF/5 cell lines, which FGF19 has been proved to upregulate OATP3A1 expression via specificity protein 1 (SP1) and NF-κB signaling (6). However, whether and how TNFα regulates OATP3A1 expression in human cholestasis is unclear.

Here, we show that hepatic OATP3A1 is positively correlated with plasma TNFα levels higher in cholestatic patients; further, we identify that TNFα increased OATP3A1 expression by activation of NF-κB p65 and extracellular signal–regulated kinase (ERK)–SP1 signaling. Our data illustrate the molecular mechanism about the upregulation of OATP3A1 induced by TNFα and gain more insight into the significance of OATP3A1 as a potential therapeutic target in cholestasis.
OATP3A1 upregulated by TNFα-activated NF-κB/ERK signaling

Results

The elevation of plasma TNFα levels positively correlated with hepatic OATP3A1 mRNA levels in cholestatic patients

We have found that OATP3A1 levels in hepatic tissues were higher in cholestatic patients compared with control patients (4.8-fold and 3.6-fold higher OATP3A1 protein and mRNA levels, respectively) (6). RT–quantitative real-time PCR (RT–qPCR) analysis showed that liver TNFα mRNA expression in obstructive cholestatic patients was significantly increased relative to the control (Fig. 1A). The elevated protein levels and serum levels of TNFα were confirmed in obstructive cholestatic patients (Fig. 1, B and C), and the linear regression analysis represented that hepatic OATP3A1 expression positively correlated with plasma TNFα levels (Fig. 1D). These results indicated that TNFα may contribute to induction of OATP3A1 expression in obstructive cholestatic patients.

Recombinant TNFα stimulated OATP3A1 expression in vitro

Next, we assessed the effect of TNFα-stimulated OATP3A1 expression. Dual-luciferase reporter analysis revealed that both FGF19 and TNFα enhanced SLCO3A1 promoter activity, and TNFα upregulated the transcriptional activity of the SLCO3A1 promoter (~2000 to +25 relative to the putative transcription start site) more obviously than FGF19 (Fig. 2A), suggesting that TNFα has a vital role in OATP3A1 transcriptional regulation. We treated PLC/PRF/5 cells and primary mouse hepatocytes with recombinant TNFα and found that it increased OATP3A1 expression in a dose-dependent manner. RT–qPCR analysis revealed that treatment with 100 ng/ml TNFα for 24 h, respectively, elevated OATP3A1 mRNA levels by 8.1-fold in primary mouse hepatocytes and 3.2-fold in PLC/PRF/5 cells relative to the control (Fig. 2, B and C), with no effect on cell viability of PLC/PRF/5 cells (Fig. S1A). Meanwhile, RT–qPCR analysis confirmed the similar facilitating effect of TNFα on OATP3A1 upregulation in Huh7 and HepG2 cells (Fig. S1, B and C). Consistent with this, cell surface protein levels of OATP3A1 were elevated dose-dependently and time-dependently relative to the control (Fig. 2, D–F). All these results revealed that TNFα could promote the expression of OATP3A1 by increasing the transcriptional activity of SLCO3A1.

TNFα increased nuclear NF-κB p65 and SP1 expression and the binding activities of p65 and SP1 to SLCO3A1 promoter

Given that FGF19 induced adaptive expression of OATP3A1 via ERK/NFκB–SP1/p65 pathways in cholestatic liver (6), we asked if NF-κB p65 and SP1 promote OATP3A1 transcriptional activity upon TNFα treatment. As expected, the nuclear protein levels of NF-κB p65 and SP1 were enhanced in primary mouse hepatocytes treated by TNFα in a dose-dependent manner (Fig. 3A), and these were elevated in PLC/PRF/5 cells in time-dependent and dose-dependent manners (Fig. 3, B and C). For further determination, SLCO3A1 promoter deletion-luciferase constructs were generated to identify the cis-acting elements respond to NF-κB p65 and SP1 overexpression and TNFα treatment in PLC/PRF/5 cells (Fig. 3D). TNFα-induced luciferase activity of the −427

Figure 1. TNFα level is elevated in hepatic tissues of obstructive cholestatic patients (n = 16). A, hepatic TNFα mRNA level versus the control group (n = 15). B, Western blot images for TNFα and their densitometric values were normalized to the control group (C1–4, control; O1–4, obstructive cholestasis livers). C, plasma levels of TNFα in cholestatic patients and control. Data are shown as mean ± SD. *p < 0.05 versus control. The data were analyzed with independent-samples Student’s t test (two-tailed). D, linear regression analysis for plasma TNFα levels and hepatic induction of OATP3A1 mRNA levels in obstructive cholestatic patients (n = 16). OATP3A1, organic anion–transporting polypeptide 3A1; TNFα, tumor necrosis factor α.
to +25 SLC03A1 promoter region (pGL3-SLC03A1-427) containing NF-κB p65 cis-acting element, respectively, increased by 20-fold and 10-fold in the presence or the absence of NF-κB p65 overexpression, relative to the −183 to +25 SLC03A1 promoter region (pGL3-SLC03A1-183) (Fig. 3E). In addition, TNFα-induced luciferase activity of the −3478 to +25 SLC03A1 promoter region (pGL3-SLC03A1-3478) correspondingly increased by 2.4-fold and 1.6-fold with or without SP1 overexpression, relative to the −2578 to +25 SLC03A1 promoter region (pGL3-SLC03A1-2578) (Fig. 3F). Taken together, these data indicated that NF-κB p65 cis-acting elements located between −427 and −183 contributed to enhanced SLC03A1 promoter activity, whereas SP1 cis-acting elements situated between −3478 and −2578 of SLC03A1 promoter.

To further identify the key sites of NF-κB p65 and SP1 regulating SLC03A1 transcription, we performed dual-luciferase reporter assays using mutated NF-κB p65 and SP1 core site plasmids described in our previous study (6). As showed, relative to pGL3-SLC03A1-427 promoter, NF-κB p65 cotransfection with pGL3-SLC03A1-427MUT promoter completely abolished dual-luciferase activity (Fig. 3G), whereas cotransfection of pGL3-SLC03A1-3478MUT construct with SP1 reduced dual-luciferase activity by 3.3-fold, compared with pGL3-SLC03A1-3478 promoter (Fig. 3H).

Furthermore, chromatin immunoprecipitation (ChIP) assay was used to determine if NF-κB p65 and SP1 could directly interact with potential binding sites on SLC03A1 promoter and whether the binding activity of NF-κB p65 and SP1 was enhanced by TNFα treatment. ChIP assay revealed that direct binding activity of NF-κB p65 and SP1 was markedly elevated by TNFα in a dose-dependent manner, representing in ChIP 2-NF-κB p65 site located at −427 to −183 and ChIP 4-SP1 site located at −3478 to −2578, respectively (Fig. 3I and J). The TNFα-induced binding activity of these transcription factors was no observable difference relative to the control, respectively, showing in the ChIP 1-NF-κB p65 site located at −643 to −427 and ChIP 3-SP1 site situated at −3689 to −3478 (Fig. 3I, J and K). ChIP assays confirmed that TNFα promotes the direct interaction of NF-κB p65 and SP1 with the SLC03A1 promoter. Together, these findings offer solid evidence that TNFα upregulated SLC03A1 expression by upregulating NF-κB p65 and SP1 transcription factors binding to the cis-acting elements of SLC03A1 promoter.
OATP3A1 upregulated by TNFα-activated NF-κB/ERK signaling

Figure 3. TNFα elevated nuclear protein levels of NF-κB p65 and SP1 as well as p65 and SP1 binding to the OATP3A1 promoter. A–C, TNFα increased SP1 and NF-κB p65 nuclear protein expression dose-dependently and time-dependently in primary mouse hepatocytes and PLC/PRF/5 cells. D, the schematic diagram of SLCO3A1 promoter and construction of deletion mutant luciferase reporter plasmids. E and F, induction of SLCO3A1 promoter activity by NF-κB p65 or SP1 constructs was markedly enhanced by TNFα (100 ng/ml). G and H, dual-luciferase reporter assays for mutated NF-κB p65 and SP1 core site plasmids of the SLCO3A1 promoter. I and J, ChIP assay results showed that TNFα elevated NF-κB p65 and SP1 binding to their response elements on the SLCO3A1 promoter in a dose-dependent manner. Data are shown as mean ± SD and normalized to the control group (0 ng/ml). *p < 0.05 versus designated group. The data were analyzed with one-way ANOVA test. ChIP, chromatin immunoprecipitation; OATP3A1, organic anion–transporting polypeptide 3A1; PLC, peritoneal lavage cell; SP1, specificity protein 1; TNFα, tumor necrosis factor α.
TNFα activated NF-κB and ERK signaling in human hepatoma PLC/PRF/5 cells

TNFα engages in several signaling pathways, for example, ERKs and the p38 mitogen-activated protein kinase signaling (17, 18). Our previous work showed that FGF19 induces adaptive OATP3A1 upregulation by activating ERK and NF-κB p65 signaling in cholestatic liver (6). We assessed if NF-κB and ERK signaling are activated by TNFα in PLC/PRF/5 cells. Relative to the control, NF-κB p65 protein phosphorylation in PLC/PRF/5 cells increased upon treatment with TNFα, and similar results were obtained for ERK1/2 phosphorylation (Fig. 4). TNFα promoted NF-κB p65 and ERK1/2 phosphorylation dose-dependently and time-dependently (Fig. 4, A and B). These results indicated that NF-κB and ERK signaling may be involved in regulation of OATP3A1 induced by TNFα.

TNFα induced OATP3A1 expression through NF-κB and ERK signaling—stimulated nuclear p65 and SP1 expression

To gain further insight into the mechanism underlying OATP3A1 regulation by TNFα, we assessed if TNFα–NF-κB/ERK–p65/SP1 signaling can be activated in PLC/PRF/5 cells. Thus, we pretreated cells with BAY 11-7082 (10 μmol/l), an irreversible and selective NF-κB signaling inhibitor, and with PD98059 (25 μmol/l), an ERK signaling blocker, before TNFα treatment. Relative to the control, we found that TNFα alone elevated the cell surface levels of OATP3A1 protein, which was suppressed by pretreatment with BAY 11-7082 or PD98059 in primary mouse hepatocytes and PLC/PRF/5 cells (Fig. 5, A and B) as well as OATP3A1 mRNA levels in Huh7 and HepG2 cells (Fig. S2, A and B). Consistent with these changes, TNFα–elevated nuclear protein levels of NF-κB p65 and SP1 were diminished by pretreatment with BAY 11-7082 or PD98059 in primary mouse hepatocytes (Fig. 5C). Moreover, NF-κB p65 and ERK1/2 protein phosphorylation was impaired by pretreatment with the NF-κB or ERK inhibitor relative to the TNFα–only treatment in PLC/PRF/5 cells (Fig. 5D), and these data further imply the presence of signaling crosstalk between NF-κB and ERK signaling. Therefore, these results delineate that NF-κB signaling mainly promoted TNFα–induced p65 expression, whereas ERK cascades mainly regulated TNFα–stimulated SP1 expression.

To further determine if NF-κB p65 and SP1 trans–acting factors could directly bind to SLCO3A1 promoter, ChIP assays were performed and revealed that TNFα–induced NF-κB p65 (ChIP 2) and SP1 (ChIP 4) binding activities were correspondingly suppressed by the NF-κB inhibitor (BAY 11-7082) and ERK inhibitor (PD98059) (Fig. 5, E and F), and these data were consistent with the results in Figure 3, I and J. Thus, our findings showed that TNFα triggered NF-κB p65 and ERK–SP1 signaling pathways and induced transcriptional upregulation of OATP3A1 (Fig. 5G).

Discussion

Here, we show a new regulatory mechanism of OATP3A1 in human cholestasis. The three main findings are increased plasma levels of TNFα significantly correlated with OATP3A1 upregulation in livers of obstructive cholestasis patients (Fig. 1D); recombinant TNFα elevated OATP3A1 expression in primary mouse hepatocytes and hepatoma cells (Figs. 2, B–F and S2); and recombinant TNFα increased p65 and SP1 binding to the SLCO3A1 promoter via NF-κB and ERK signaling pathways (Figs. 3–5).

Cholestasis refers to a spectrum of liver diseases characterized by accumulation of potentially toxic BAs. The impaired BA excretion and homeostasis results in cholestasis-related liver damage (1). In cholestasis, upregulation of BA efflux transporters, for example, MRP3 and OATP3A1 enhance BA elimination by hepatocytes (2–6). OATP3A1 deletion in mice aggravated BA accumulation and cholestatic liver injury, indicating that OATP3A1 has a pivotal role in cholestasis (6). We previously reported that FGF19 stimulates OATP3A1 expression in hepatocytes by activating ERK and NF-κB

Figure 4. TNFα activated NF-κB and ERK signaling in human hepatoma PLC/PRF/5 cells. A and B, TNFα increased phosphorylation levels of NF-κB p65 and ERK1/2 dose-dependently and time-dependently. TNFα induced total NF-κB p65 protein expression. Data are shown as mean ± SD and normalized to control group (0 ng/ml). *p < 0.05 versus control. The data were analyzed with one-way ANOVA test. ERK, extracellular signal–regulated kinase; PLC, peritoneal lavage cell; TNFα, tumor necrosis factor α.
OATP3A1 upregulated by TNFα-activated NF-κB/ERK signaling

Figure 5. TNFα–NF-κB/ERK–p65/SP1 signaling pathway mediated OATP3A1 expression in PLC/PRF/5 cells and primary mouse hepatocytes treated by TNFα with or without the NF-κB and ERK signaling inhibitors BAY11-7082 (10 μmol/l) and PD98059 (25 μmol/l). A and B, biotinylated cell surface protein (OATP3A1) was detected by Western blot in primary mouse hepatocytes and PLC/PRF/5 cells, respectively. C, Western blot detection of NF-κB p65 and SP1 expression in nuclear extracts of primary mouse hepatocytes. D, the expression of phosphorylated NF-κB p65 and SP1 was detected by Western blot in PLC/PRF/5 cells. E and F, NF-κB p65 and SP1 cis-acting elements on SLCO3A1 promoter were further detected by ChIP assays, and corresponding quantifications were shown below these gels. All experiments were repeated for three times. Data are shown as mean ± SD and normalized to control group without treatment. *p < 0.05 versus control, #p < 0.05 versus TNFα-treated group. The data were analyzed with one-way ANOVA test. G, the schematic diagram of the mechanism for TNFα-induced OATP3A1 expression. ChIP, chromatin immunoprecipitation; ERK, extracellular signal–regulated kinase; OATP3A1, organic anion–transporting polypeptide 3A1; PLC, peritoneal lavage cell; SP1, specificity protein 1; TNFα, tumor necrosis factor α.
signaling in cholestasis. We also found that TNFα induced OATP3A1 mRNA expression in hepatoma PLC/PRF/5 cells (6). However, whether and how TNFα promote OATP3A1 levels in hepatic cells in cases of obstructive cholestasis is unclear. Here, we find that elevated levels of plasma TNFα significantly correlate with hepatic OATP3A1 mRNA expression in obstructive cholestasis patients (Fig. 1D), indicating that TNFα stimulates hepatic OATP3A1 expression in cholestasis. Further mechanism studies revealed that recombinant TNFα enhanced OATP3A1 protein levels in hepatocytes as well as binding activity of p65 and SP1 to OATP3A1 promoter (Figs. 2 and 3). Moreover, these alterations were suppressed by the NF-κB and ERK signaling inhibitors, BAY 11-7082 and PD98059, respectively (Fig. 5). These results implied the presence of signaling crosstalk between NF-κB and ERK when TNFα induced transcriptional upregulation of OATP3A1, and it is necessary to further investigate the more details of this mechanism in the adaptive response to cholestasis. Collectively, our findings showed that elevated TNFα levels stimulate hepatic OATP3A1 expression via NF-κB and ERK signaling in human obstructive cholestasis. Our findings highlight a novel mechanism of OATP3A1 regulation in cholestasis.

Recent studies show that excessive accumulation of intrahepatic BA triggers inflammatory response by producing inflammatory cytokines and chemokines in the cholestatic liver (19–21). BA accumulation in intestines may activate nuclear receptor farnesoid X receptor and stimulate FGF19 expression (22). BA also stimulates TNFα expression in PLCs (23). Elevated plasma levels of TNFα and FGF19 have been observed in human cholestasis (2, 6). Elevated serum TNFα and FGF19 can target cholestatic liver, repressing BA synthesis and FGF19 in cholestasis. Our findings uncovered a new regulatory mechanism of OATP3A1 as a BA efflux transporter in human cholestasis. Thus, we surmise that TNFα-activated NF-κB p65 and ERK–SP1 signaling may be a potential target to mitigate cholestasis-associated liver injury.

**Experimental procedures**

**Patients and liver sample collection**

Ethical approval for the study was granted by the Institutional Ethics Review Committee, Southwest Hospital in Chongqing, China. All patients signed written informed consent. Cholestatic liver samples (n = 16) were collected from patients suspected with periampullary or pancreatic malignancy with severe obstructive cholestasis manifestations. Control group also received liver tissue from patients who underwent liver metastatic resection without cholestasis (n = 15). Clinical features of patients are listed as described previously (6).

**Cell culture and treatment**

Primary mouse hepatocytes were collected through liver perfusion of C57BL/6J mice with collagenase; human hepatocellular carcinoma PLC/PRF/5, HepG2, and HuH7 hepatocellular carcinoma cell lines were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum in a 5% CO₂ with 37 °C humidified incubator. Before chemical treatment, the cells were starved by the Dulbecco’s modified Eagle’s medium with 2% serum (fetal bovine serum) for 12 h, and then TNFα was treated with a gradient concentration (0, 10, 30, and 100 ng/ml), or with 100 ng/ml for 24 h or time gradient (0, 1, 3, 6, 12, and 24 h), inhibitors were pretreated 30 min prior to treatment. TNFα was obtained from PeproTech. PD98059 and BAY 11-7082 were obtained from Sigma–Aldrich. Experiments related to animals were approved by the Institutional Animal Care and Use Committee of the Southwest Hospital affiliated with the Third Military Medical University. Cell viability was assessed by Cell Counting Kit-8 (Genview) according to the manufacturer’s protocols.

**RNA extraction and RT–qPCR**

Total RNA was extracted from liver tissues or cultured cells with Trizol reagent (Invitrogen), then reversely transcribed into complementary DNA (cDNA) with cDNA Synthesis Kit (MBI Fermentas, Inc). The collected cDNA was used for RT–qPCR SYBR premix Ex Taq II Kit (Takara Biotechnology) in a Bio-Rad CFX96. The primer sequences are listed in Table 1, and GAPDH/Gapdh was used as a loading control.

Table 1

<table>
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<th>Gene</th>
<th>Species/source</th>
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<td>GAPDH</td>
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<td>OATP3A1 (SLCO3A1)</td>
<td>Human/Hs0203194_m1</td>
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<td>Mouse/primers (SYBR); NM_001289726.1</td>
<td>Forward: 5’-ACC CTT AAG AGG GAT GCT GC-3’ Reverse: 5’-CGG GAC GAG GAA ACA CTC TC-3’</td>
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<td>Oatp3a1 (Slco3a1)</td>
<td>Mouse/primers (SYBR); NM_001038643.1</td>
<td>Forward: 5’-CAG TGC CCG AAA AAC TAT T-3’ Reverse: 5’-GAC TCA GGG CAG GTT TTC TCT-3’</td>
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The radioimmunoprecipitation assay buffer (Sigma) containing phosphatase and protease inhibitors (Roche) was used for extracting total protein. The commercial kits from Thermo Fisher Scientific were used for extracting nuclear extraction and cell surface protein biotinylation (2). Protein was separated by SDS–PAGE. Primary antibody sources and dilutions are listed in Table 2.

### ELISA for detection of TNFα levels in plasma

Plasma samples (control patients, n = 15, and obstructive cholestatic patients, n = 16) were collected before biopsy or hepatectomy using heparin and stored at −80 °C. Levels of TNFα in the collected samples were quantified using TNFα ELISA Kit (BD Biosciences) according to the manufacturer’s instructions.

### ChIP assay

ChIP assays were conducted using a commercial ChIP Assay Kit (Millipore) according to the manufacturer’s instructions. PLC/PRF/5 cells were used for preparing soluble chromatin. The chromatins were immunoprecipitated using antibodies against SP1 and NF-κB p65 (Table 2). Next, the specific regions containing SP1 or NF-κB p65 binding sites of SLCO3A1 promoter were amplified with primers described in our previous study (30), which the ChIP1 (−623) and ChIP2 (−383) were for NF-κB p65 binding sites and ChIP3 (−3629) and

### Table 2

**Antibodies used in Western blot (WB) and ChIP**

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<td>SP1 (H-225)</td>
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<td>Santa Cruz/sc-14027</td>
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### Table 3

**The primer sequences used for construction of SLCO3A1 promoter plasmids**

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<td>Forward: 5’-CGACCGGT TCA ACA GAA GCA GCA AAT G-3’&lt;br&gt;Reverse: 5’-GAAGATC T CCG CCG CCG CCG CCG CCA TC-3’</td>
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<td>pGL3-SLCO3A1-3689</td>
<td>Forward: 5’-CGACCGGT GCT GGG GCT GAG GGG GTG AG-3’&lt;br&gt;Reverse: 5’-GAAGATC T CCG CCG CCG CCG CCG CCA TC-3’</td>
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<tr>
<td>pGL3-SLCO3A1-3478</td>
<td>Forward: 5’-CGACCGGT GCC TCC CCG CAG CAC GAT GAG G-3’&lt;br&gt;Reverse: 5’-GAAGATC T CCG CCG CCG CCG CCG CCA TC-3’</td>
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<td>Forward: 5’-CGACCGGT GCC TCC CCG CAG CAC GAT GAG G-3’&lt;br&gt;Reverse: 5’-GAAGATC T CCG CCG CCG CCG CCG CCA TC-3’</td>
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<td>pGL3-SLCO3A1-183</td>
<td>Forward: 5’-CGACCGGT GCC TCC CCG CAG CAC GAT GAG G-3’&lt;br&gt;Reverse: 5’-GAAGATC T CCG CCG CCG CCG CCG CCA TC-3’</td>
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ChIP4 (~3453) were for SP1 binding sites. The PCR products were used for agarose gel electrophoresis to detect the levels of SLCO3A1 promoter motif binding to SP1 and NF-κB p65. The primer sequences are listed in Table 4.

### Statistical analysis

The GraphPad Prism (version 6.01) (GraphPad Prism Software) was used for data analysis. Data were shown as mean ± SD and analyzed with one-way ANOVA test and independent-samples Student’s t test (two-tailed). p < 0.05 indicated statistical significance.

### Data availability

All experimental data for this article are available upon e-mail request to Qiong Pan (Southwest Hospital, qiong.pan@cldcswe.org).

**Supporting information—**This article contains supporting information.

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**Conflict of interest—**The authors declare that they have no conflicts of interest with the contents of this article.

**Abbreviations—**The abbreviations used are: BA, bile acid; cDNA, complementary DNA; ChIP, chromatin immunoprecipitation; ERK, extracellular signal–regulated kinase; FGF19, fibroblast growth factor 19; MRP3, multidrug resistance–associated protein 3; OATP3A1, organic anion–transporting polypeptide 3A1; PLC, plinelineal lavage cell; RT–qPCR, RT–quantitative real-time PCR; SP1, specificity protein 1; TNFα, tumor necrosis factor α.

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