Available online at www.sciencedirect.com

SciVerse ScienceDirect

Journal of Nutritional Biochemistry 23 (2012) 785–790

Journal of Nutritional Biochemistry

Liver X receptor negatively regulates fibroblast growth factor 21 in the fatty liver induced by cholesterol-enriched diet☆

Takashi Uebanso[a,1], Yutaka Taketani[a,*], Hironori Yamamoto[a], Kikuko Amo[a], Sarasa Tanaka[a], Hidekazu Arai[b], Yuichiro Takei[a], Masashi Masuda[a], Hisami Yamanaka-Okumura[a], Eiji Takeda[a]

[a]Department of Clinical Nutrition, Institute of Health Biosciences, University of Tokushima Graduate School, Tokushima 770-8503, Japan
[b]Laboratory of Clinical Nutrition Management, School of Food and Nutritional Sciences, The University of Shizuoka, Shizuoka 422-8526, Japan

Received 25 January 2010; received in revised form 9 March 2011; accepted 23 March 2011

Abstract

Cholesterol homeostasis is regulated by the liver X receptor (LXR) at the transcriptional level, but it remains unknown whether LXR can affect expression levels of intrahepatic lipolysis related gene. Recent evidence has demonstrated that fibroblast growth factor 21 (FGF21) regulates hepatic lipolysis and fatty acid utilization. In the present study, we examined the role of LXR in FGF21 gene expression associated with regulation of cross-talk signals between cholesterol and triglyceride metabolism in the liver. An *in vivo* cholesterol feeding test revealed that intake of excess cholesterol increased cholesterol catabolism related gene expression as well as fatty-acid biosynthesis related gene expression. Moreover, the accumulated cholesterol suppressed FGF21 and hormone-sensitive lipase (HSL) gene expression. After 15-day cholesterol feeding, hepatic triglyceride concentrations were negatively correlated with expression levels of the FGF21 and HSL genes in the liver. An LXR agonist (TO-901317) repressed the FGF21 gene expression in mouse primary hepatocytes and HepG2 cells. A promoter deletion study and electrophoretic mobility shift assay revealed that the human FGF21 promoter has at least one LXR response element located from −37 to −22 bp. In summary, LXR represses FGF21 gene expression at the transcription level and might suppress lipolysis and lipid utilization to protect the liver from excess accumulation of toxic cholesterol.

Keywords: Fibroblast growth factor 21; Liver X receptor; Lipolysis; Cholesterol; Hormone-sensitive lipase

1. Introduction

Hepatic cholesterol metabolism is closely related to triglyceride (TG) metabolism [1]. The daily cholesterol balance is regulated by three major pathways involving biosynthesis, absorption from intestine and elimination into bile [2]. Excess cholesterol feeding reduces absorption from intestine and increases catabolism to bile acids by increasing levels of cholesterol 7α-hydroxylase (CYP7a1), the rate-limiting enzyme, in the liver [3]. In addition, TG synthesis from acetyl-CoA is up-regulated to reduce cholesterol biosynthesis under these conditions and leads to TG accumulation in the liver [1,3]. Indeed, expression levels of lipogenic genes such as sterol regulatory element binding protein 1 and stearoyl-CoA desaturase 1 (SCD1) were increased in mouse fed a high-cholesterol diet [1,3].

Cholesterol homeostasis is controlled by these metabolic changes, which are in turn regulated by the liver X receptors (LXRα and LXRβ) and farnesoid X receptor (FXR) at the transcriptional level [2]. Liver X receptors were defined as sterol sensors, as they can be activated by cholesterol-derived oxysterols [2]. Farnesoid X receptor was defined as bile acid sensor and allowed an elegant model to explain the mechanism of feedback regulation of CYP7a1 by bile acid [2,4]. The LXRs and FXR form obligate heterodimers with retinoid X receptor (RXR) and regulate gene transcription for binding specific DNA element as described above [1–8].

Fibroblast growth factor 21 (FGF21) has recently emerged as a metabolic hormone involved in the regulation of glucose and lipid metabolism [9–12]. In particular, FGF21 regulates lipolytic genes and stimulates fatty acid utilization for energy and ketone body production in the liver [11]. Fibroblast growth factor 21 knockdown mice show accelerated hepatic TG accumulation when fed a ketogenic diet [9]. Moreover, FGF21 stimulates fatty acid oxidation and protects from diet-induced obesity and metabolic disorders, such as fatty liver,

☆ This work was supported by Grants-in-Aid for Scientific Research for Young Scientists (18790567) and for Scientific Research (B) (18300232, 22300237) from the Ministry of Education, Culture, Sports, Science, and Technology in Japan, the 21st Century COE program "Human Nutritional Science and Stress Control", and the Initiatives for Attractive Education in Graduate School from the University of Tokushima, Tokushima, Japan.

* Corresponding author. Department of Clinical Nutrition, School of Medicine, University of Tokushima, Tokushima 770-8503, Japan. Tel.: +81 886 33 7095; fax: +81 886 33 7094.
E-mail address: taketani@nutr.med.tokushima-u.ac.jp (Y. Taketani).
[1] Present address: Department of Metabolic Disorder, Diabetes Research Center, Research Institute, National Center for Global Health and Medicine, Tokyo 162-8655, Japan.

0955-2863/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2011.03.023

in obese or diabetic animals given FGF21 [10,12]. These data indicate that FGF21 regulates hepatic TG levels through intrahepatic lipolysis and fatty acid utilization. However, it remains unknown whether excess cholesterol feeding can affect intrahepatic TG lipolysis and lipid utilization or not.

In the present study, we have investigated whether LXR and/or FXR can transcriptionally regulate intrahepatic lipolysis and lipid utilization related genes, especially FGF21. An *in vivo* cholesterol feeding test revealed reduction of lipolysis and lipid utilization related genes, including FGF21 and hormone-sensitive lipase (HSL), in the mouse liver. The FGF21 gene expression was negatively regulated by LXR in HepG2 cells and mouse primary hepatocytes. Thus, this study will aid in understanding the cross-talk between cholesterol and triglyceride metabolism, especially lipolysis and lipid utilization in the liver.

2. Materials and methods

2.1. Animals

Male ddy mice from a local breeding colony (Japan SLC, Shizuoka, Japan) were used in all experiments. Mice were housed in cages maintained at constant temperature (23°C ±2°C) and humidity (65%–75%) with a 12-h light (8:00–20:00), 12-h dark cycle. Prior to the study, mice were allowed free access to tap water and chow, a standard laboratory rodent diet (MF; Oriental Yeast, Osaka, Japan) consisting of 54.4% carbohydrate, 23.6% protein and 5.3% fat (% total energy), with an energy density of 15.0 kJ/g.

Male 6-week-old ddy mice were divided into two dietary groups: The control group was fed chow (MF) and tap water *ad libitum* for 5 (n=8) or 15 days (n=9); the cholesterol group was fed MF supplemented with 2% cholesterol and tap water *ad libitum* for 5 (n=8) or 15 days (n=9). All mice were killed at 8:00 for collection of blood, liver and visceral adipose tissue. Prior to sacrifice, a blood sample from the tail vein was used to determine plasma glucose concentrations. The University of Tokushima Animal Use Committee approved the study, and mice were maintained according to the National Institutes of Health guidelines for care and use of laboratory animals.

2.2. Plasma glucose, lipid and hepatic lipid concentrations

The plasma glucose concentration was measured by the glucose dehydrogenase method using an Accu-Chek blood glucose meter (Roche Diagnostics). Plasma TG, total cholesterol and nonesterified fatty acid (NFFA) concentrations were measured by using Triglyceride-E, Cholesterol-E and NEFA-C tests (Wako Pure Chemical Industries), respectively. Hepatic lipids were extracted and measured as previously described [13].

2.3. RNA preparation and quantitative reverse transcriptase polymerase chain reaction

Extraction of total RNA, cDNA synthesis and real-time polymerase chain reaction (PCR) analysis were performed as described previously [13]. The relative abundance of each target transcript (primers sequences in Table 1) was calculated by normalization to the amount of amplified product from constitutively expressed β-actin mRNA.

2.4. Plasmids

Polymerase chain reaction was performed with an Expand High-Fidelity PCR system (Roche) and primers (Invitrogen). Luciferase reporter vector constructs containing −1672 to +230 bp of the human FGF21 promoter (pFGF21-1.6k) and a series of 5′-deletion mutant vectors (pFGF21-1k, pFGF21-555, pFGF21-289, pFGF21-83 and pFGF21+11) were prepared from human genomic DNA and pGL4.12 vectors (Promega). The expression plasmid vectors, pcDNA (Invitrogen)-mouse LXRα and LXRβ, were prepared by subcloning PCR products from mouse liver cDNA into pcDNA3.1. The mouse RXR expression vector (pSG5-mRXRα) was kindly provided by Prof. P. Chambon.

Table 1
Sequence of oligonucleotide primers for quantitative reverse transcriptase PCR analysis

Gene name	Accession no.	Primer sequence
β-Actin (human)	NM_001101	F: 5′-GGCACCACACCTTCTACAATGAGC-3′ R: 5′-AGCCTGGATAGCAACGTACATGGC-3′
β-Actin (mouse)	NM_007393	F: 5′-CTGACCCTGAAGTACCCCATTGAACA-3′ R: 5′-CTGGGGTGTTGAAGGTCTCAAACATG-3′
FGF21 (human)	NM_019113	F: 5′-GGGAGTCAAGACATCCAGGT-3′ R: 5′-GGCTTCGGACTGGTAAACAT-3′
FGF21 (mouse)	NM_020013	F: 5′-CTACCAAGCATACCCCATCC-3′ R: 5′-GCCTACCACTGTTCCATCCT-3′
CYP7a1 (mouse)	NM_007824	F: 5′-GAGCCCTGAAGCAATGAAG-3′ R: 5′-GCTGTCCGGATATTCAAGGA-3′
SCD1 (mouse)	NM_009127	F: 5′-CCACATGCTCCAAGAGATCTCCAGTTC-3′ R: 5′-GTTCCTCCAGACGTACTCCAGCTTGG-3′
HSL (mouse)	NM_010719	F: 5′-ACAGAGGCAGAGGACCATTT-3′ R: 5′-CTTGCGTCCACTTAGTTCCA-3′

2.5. Cell culture

Primary hepatocytes were isolated from normal male ddy mice (20–25 g) by using the collagenase perfusion method as described previously [13]. Hepatocyte suspensions were plated on 35-mm plastic dishes in a final volume of 2 ml of William's E medium (Sigma) supplemented with 1 nmol of insulin (Sigma), 1 nmol of dexamethasone (Sigma), 10% (v/v) fetal bovine serum (Invitrogen) and 1% (v/v) penicillin/streptomycin (Sigma). After 6 h of attachment, the medium was removed, and fresh serum-free medium was added. After 12 h in culture, the cells were stimulated for the indicated time with medium containing TO-901317 (Sigma) or GW4064 (Sigma).

Human hepatoma HepG2 and opossum kidney OK cells were cultured in Dulbecco's modified Eagle's medium (Sigma) containing 10% (v/v) fetal bovine serum (Invitrogen) and 1% (v/v) penicillin/streptomycin (Sigma).

2.6. Transfections and luciferase assays

Transfection studies were carried out in primary hepatocytes and OK (opossum kidney) cells. The indicated amounts of each expression plasmid were transfected simultaneously with a luciferase reporter plasmid (0.4–1.0 μg), pCMV-β-galactosidase (0.2–0.5 μg, CLONTECH), pcDNA-mLXRα/mLXRβ (0.2 μg) and/or pSG5-mRXRα (0.2 μg) with Lipofectamine 2000 (Invitrogen). The total amount of DNA in each transfection was adjusted to 1.5 μg/well with pCMV. After 4 h of transfection, the cells were cultured with medium containing TO-901317 for 20 h, and the amount of luciferase activity in the transfectants was measured and normalized to the amount of β-galactosidase activity as measured by a standard kit (Promega).

2.7. Electrophoretic mobility shift assays

Electrophoretic mobility shift assays were performed as previously described [14]. In brief, mLXRα and mRXRα proteins were generated from the expression vectors by using a coupled *in vitro* transcription/translation system (Promega). Human FGF21 promoter LXR element (LXRE) double-stranded oligonucleotides were generated, annealed and labeled with [γ-^{32}P]-ATP. The labeled probes were incubated with nuclear proteins and 2 μg of poly (dI-dC) in binding buffer. The DNA–protein complexes were resolved on a 5% polyacrylamide gel electrophoresis gel at 150 V for 90 min. The gels were dried and analyzed with BAS3000 (Fuji Photo Film).

2.8. Statistical analyses

All values are expressed as mean±S.E. The significance of differences between two groups was assessed by using unpaired two-tailed t test. For comparison between more than two groups, we employed analysis of variance (ANOVA) or the Kruskal–Wallis test. When a significant difference was found by ANOVA or the Kruskal–Wallis test, post hoc analyses were performed by using the Tukey–Kramer protected least significant difference test. Concentration-dependent effects were found by using regression analysis. Spearman's rank correlation coefficient was used to calculate

Table 2
Body weight, organ weight, liver and plasma parameters of mice fed the cholesterol diet for 5 or 15 days

Diet	5-day feeding		15-day feeding	
	Control (n=8)	Cholesterol (n=8)	Control (n=9)	Cholesterol (n=9)
Initial BW (g)	36.6±1.3	36.8±1.0	37.3±1.0	36.8±0.8
Final BW (g)	37.6±1.0 ^{a}	38.8±0.8 ^{a}	44.5±1.0 ^{b}	45.8±0.8 ^{b}
Visceral fat (g/kg BW)	28.4±2.0 ^{a}	28.2±2.4 ^{a}	43.9±4.2 ^{b}	40.5±2.8 ^{b}
Liver parameters				
Liver (g/kg BW)	51.6±1.1 ^{a}	57.8±1.4 ^{b}	49.6±0.8 ^{a}	60.1±0.9 ^{b}
Total cholesterol (mmol/liver)	5.8±0.6 ^{a}	10.9±1.5 ^{b}	4.9±0.2 ^{a}	13.6±0.7 ^{b}
Triglyceride (mmol/liver)	7.4±1.4 ^{a}	15.7±2.7 ^{b}	9.3±1.1 ^{a}	25.5±1.6 ^{c}
Plasma parameters				
Glucose (mmol/L)	10.0±0.7	9.1±0.5	10.5±0.5	10.4±0.4
Total cholesterol (mmol/L)	2.9±0.2 ^{a}	4.3±0.2 ^{b}	3.8±0.3 ^{a,b}	4.2±0.3 ^{b}
Triglyceride (mmol/L)	1.2±0.1	1.6±0.2	1.4±0.1	1.3±0.3
NEFA (mEq/L)	0.42±0.02	0.41±0.02	0.40±0.04	0.43±0.03

Data represent mean±S.E. (n=8–9). Significant differences were observed in the different letter groups, P<.05.

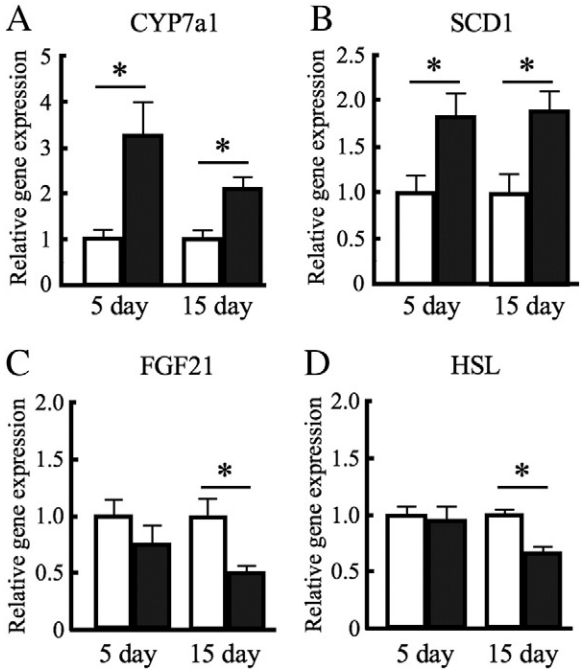

Fig. 1. Gradual changes in genetic parameters caused by cholesterol feeding in mice. Changes in expression levels of CYP7a1 (A), SCD1 (B), FGF21 (C) and HSL (D) after 5 and 15 days of cholesterol feeding. Data represent mean±S.E. (n=8–9). *P<.05. White and black bars indicate control and cholesterol diet groups, respectively.

correlation coefficients between selected variables. Differences were considered significant at P<.05. Statistical analyses were performed by using StatView (SAS) and Excel-Toukei 2006 (SSRI).

3. Results

3.1. Cholesterol feeding suppresses FGF21 gene expression in mice

We investigated whether cholesterol feeding affects FGF21 gene expression in mice because oxysterols, which are metabolites of cholesterol, are important intrinsic ligands of LXR [2]. Although there was no significant difference in the body weight (BW) and relative visceral fat weight between control and cholesterol groups, mice fed cholesterol diet showed hepatomegaly and excess accumulation of cholesterol in the liver in both 5 and 15 days of feeding (Table 2). Plasma total cholesterol concentration was higher in cholesterol-fed mice than that of control mice only in 5-day feeding (Table 2). Other plasma parameters (such as glucose, TG and NEFA levels) did not vary between groups significantly (Table 2). Consistent with the hepatic cholesterol concentration, expression of the CYP7a1 and SCD1 genes was increased by both 5 and 15 days of cholesterol feeding (Fig. 1A, B). In contrast, prolonged (15 days) feeding of the cholesterol diet was needed to strongly affect triglyceride accumulation and expression levels of the FGF21 and HSL genes in the liver (Fig. 1C, D). Moreover, after 15 days, but not 5 days, of cholesterol feeding, there was a significant and negative correlation between TG or total cholesterol concentration and FGF21 or HSL gene expression in the liver (Fig. 2A–D) in fed mice.

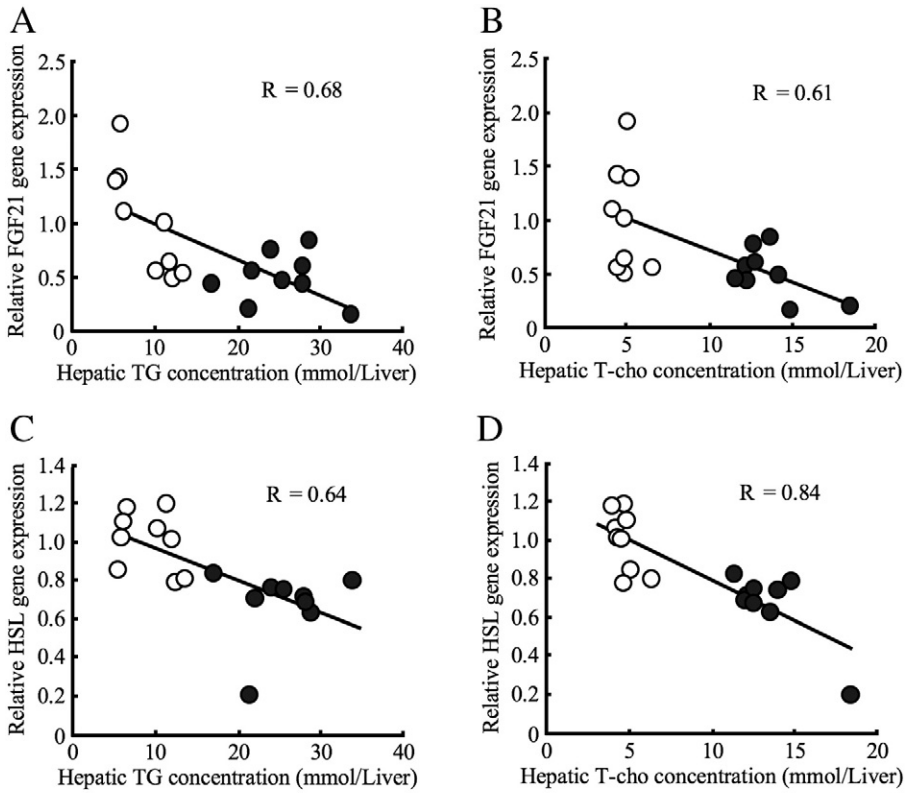

Fig. 2. Hepatic TG and total cholesterol concentration are correlated with hepatic FGF21 and HSL gene expression in mice fed the cholesterol diet for 15 days. Relationship between hepatic FGF21 and HSL gene expression and hepatic TG concentration (A, C) and total cholesterol concentration (T-cho; B, D) in mice fed the cholesterol diet for 15 days. By Spearman's rank correlation, hepatic FGF21 and HSL gene expression correlated significantly with hepatic TG concentration (FGF21: r=0.68, P=.002; HSL: r=0.64, P=.004) and with hepatic T-cho concentration (FGF21: r=0.61, P=.008; HSL: r=0.84, P<.001). White and black circles indicate the control and cholesterol diet groups, respectively.

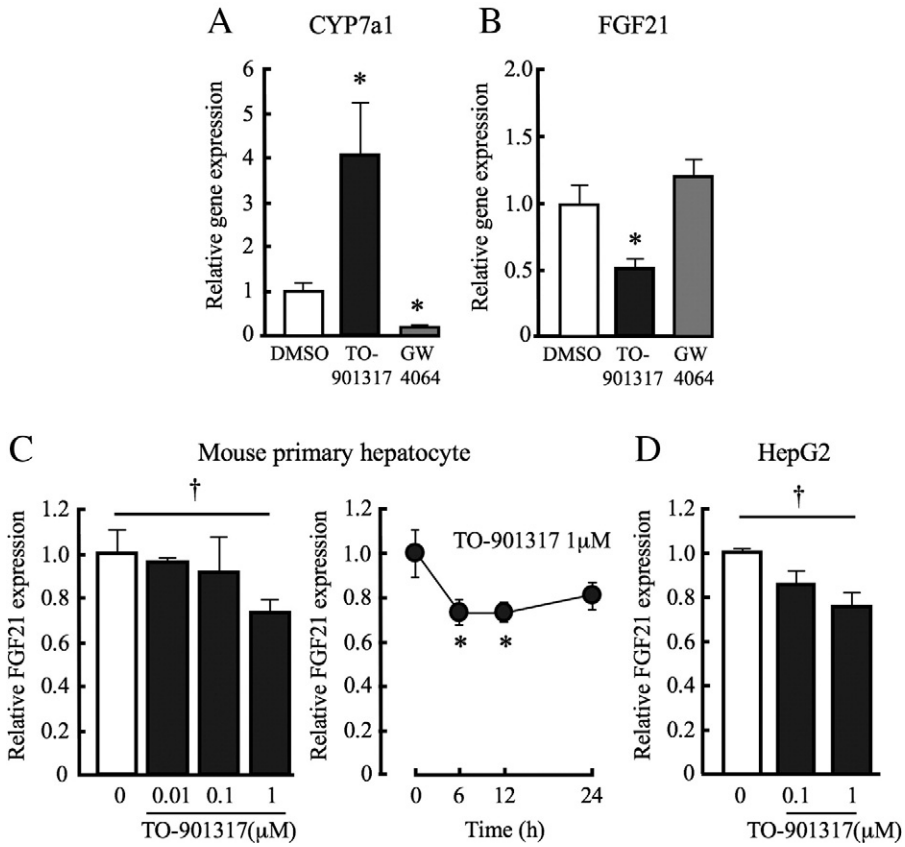

Fig. 3. Liver X receptor activation suppresses FGF21 gene expression in mouse primary hepatocytes and HepG2 cells. Changes in CYP7a1 (A) and FGF21 (B) gene expression stimulated by 1 μM TO-901317 or GW4064 for 6 h in mouse primary hepatocytes. (C) Changes in FGF21 gene expression stimulated by the indicated dose of TO-901317 for 6 h (left panel) or 1 μM TO-901317 for the indicated time (right panel) in mouse primary hepatocytes. (D) Changes in FGF21 gene expression stimulated by the indicated dose of TO-901317 for 6 h in HepG2 cells. Data represent mean±S.E. (n=3). †Concentration-dependent effects were observed by regression analysis, P<.05. *P<.05 as compared to DMSO treatment.

3.2. LXR activation leads to suppression of FGF21 gene expression

Next, we investigated how a cholesterol diet feeding suppresses hepatic FGF21 gene expression *in vitro*. Cholesterol metabolism is tightly regulated by LXR and FXR; we therefore tested the effects of LXR and FXR agonists (TO-901317 and GW4064, respectively) on FGF21 gene expression in mouse primary hepatocytes. We used CYP7a1 as a control gene for our experiments because it is well known to be up-regulated by LXR and down regulated by FXR (Fig. 3A). TO-901317 but not GW4064 treatment suppressed FGF21 gene expression in mouse primary hepatocytes (Fig. 3B). This suppressive effect was seen in a dose- and time-dependent manner, and was also observed in human hepatoma HepG2 cells (Fig. 3C–D).

3.3. LXR activation leads to suppression of human FGF21 promoter activity

To further investigate the mechanisms of repression of FGF21 mRNA expression via LXR, we constructed luciferase reporter vectors containing a series of 5′-deletions of the human FGF21 promoter. TO-901317 treatment reduced pFGF21-1.6k promoter activity in mouse primary hepatocytes, as well as gene expression (Fig. 4A). We next investigated the effects of exogenous overexpression of LXRα/LXRβ and RXRα in OK cells. Exogenous expression of mRXRα increased the FGF21 promoter activity, and this induction was suppressed by co-expression of mLXRα/mLXRβ in OK cells (Fig. 4B). TO-901317 treatment dose-dependently reduced the FGF21 promoter activity in OK cells co-expressing mLXRα/mLXRβ and mRXRα (Fig. 4C).

We then tested which region of the promoter is important for LXR-dependent suppression of the human FGF21 gene. Liver X receptor activation induced approximately 40% suppression in the deletion mutant vectors pFGF21-1.6k, pFGF21-1k, pFGF21-555, pFGF21-289 and pFGF21-83, but the pFGF21+11 mutant vector showed no response to LXR agonist stimulation (Fig. 5A). These data indicate that the human FGF21 promoter contains at least one LXR responsive element located in the region −83 to +11 bp.

3.4. The LXRα/RXRα heterodimer binds human FGF21 promoter regions

Next, we investigated whether the LXRα/RXRα heterodimer binds within the −83 to +11 bp region of the human FGF21 promoter by electrophoretic mobility shift assay. We found that the human FGF21 promoter contains a putative LXRE, composed of a direct repeat motif separated by 4-bp spaces (DR4), located in the −37 to −22 bp region (Fig. 5B). mLXRα or mRXRα alone did not bind to the −37 to −22 bp region of the human FGF21 promoter, whereas the mLXRα/mRXRα heterodimer did bind to this region (Fig. 5C). This binding was stimulated by TO-901317 pretreatment and competitively inhibited by an excess amount of a cold CYP7a1 LXRE probe.

4. Discussion

In the present study, we have focused on the regulatory mechanisms of the FGF21 gene to examine cross-talk between cholesterol and triglyceride metabolism in the liver. An *in vivo* cholesterol feeding test revealed that excess cholesterol intake

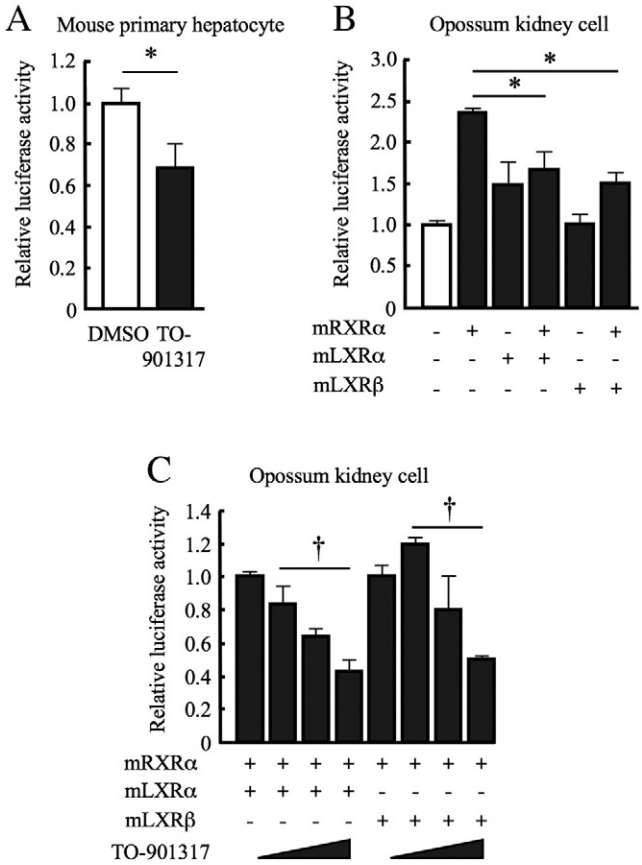

Fig. 4. Liver X receptor activation suppresses human FGF21 gene transcription in mouse primary hepatocytes and OK cells. (A) Changes in relative luciferase activity of pGL4-human FGF21 promoter reporter vectors containing −1672 to +230 bp (pFGF21-1.6k) stimulated by 1 μM TO-901317 for 6 h in mouse primary hepatocytes. (B) Changes in relative luciferase activity of pFGF21-1.6k co-expressed mLXRα/β and/or mRXRα for 24 h in OK cells. (C) Changes in relative luciferase activity of the pFGF21-1.6k vector co-expressed with mLXRα/β and/or mRXRα and either stimulated or not with 10^{-6} to 10^{-8} M TO-901317 for 20 h in opossum kidney cells. Data represent mean±S.E. (n=3). *P<.05. †Concentration-dependent effects were observed by regression analysis, P<.05.

primarily increased expression levels of the conversion of cholesterol to bile acids and fatty acid synthesis related gene, and secondarily reduced FGF21 and HSL gene expression to reduce lipolysis in the liver. Human and mouse FGF21 gene expression was negatively regulated by LXR. A deletion study and electrophoretic mobility shift assay revealed that the human FGF21 promoter has at least one LXRE in the region from −37 to −22 bp.

Liver X receptor acts as a cholesterol sensor that controls cellular cholesterol homeostasis through the transcriptional regulation of several genes [3]. We found that some LXR target genes were rapidly regulated (within 5 days of starting cholesterol feeding) and other genes were slowly regulated (within 15 days of cholesterol feeding). Expression levels of CYP7a1 and SCD1 were negatively correlated with expression levels of FGF21 and HSL in mouse fed the high-cholesterol diet for 15 days, but not for 5 days (data not shown). It is not clear why and how this difference was caused, but it might reflect the cellular response to adapt to excess accumulation of cholesterol in the liver. In particular, hepatic lipolytic genes, including FGF21 and HSL, were suppressed for keeping pace with TG accumulation in the liver. The expression levels of lipolytic genes were significantly and negatively correlated with hepatic TG concentration. Inagaki et al. have reported that FGF21 regulates lipolytic genes and that FGF21 stimulates fatty acid utilization for energy and ketone body production [11]. In contrast, FGF21 knockdown mice show accelerated hepatic TG accumulation when fed a high-fat diet [9]. We have also reported that hepatic TG concentrations were negatively correlated with hepatic FGF21 gene expression levels in obese rats fed caloric-restricted diet [13]. These data indicate that FGF21 regulates hepatic TG levels partly through intrahepatic lipid utilization. Suppression of FGF21 gene expression by excess cholesterol accumulation may reduce the supply of substrate for cholesterol biosynthesis. This suppression prevents a futile cycle between TG and fatty acids.

It has been reported that unliganded LXR can exert promoter-specific transcriptional repression, but in that case, additive suppressive effects were not observed after stimulation with LXR ligand [15]. In addition, the peroxisome proliferator-activated receptor α (PPARα) can bind the DR4 and compete against LXR-mediated

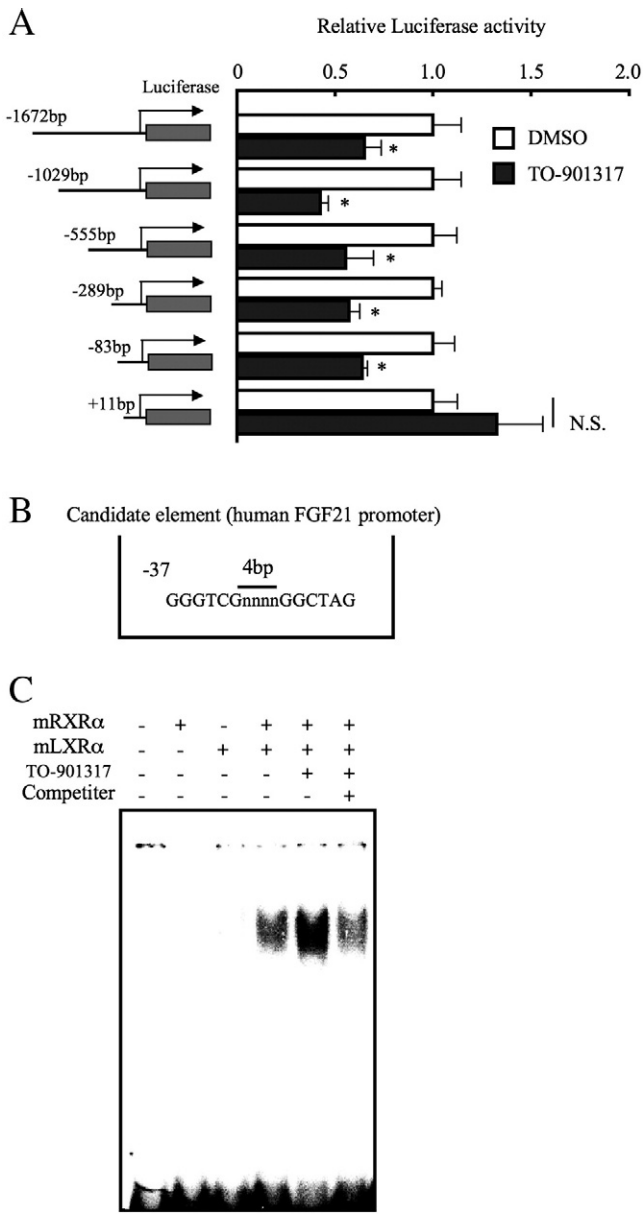

Fig. 5. The human FGF21 promoter has a putative LXR responsive element. (A) Schematic representation of the human FGF21 promoter luciferase deletion constructs and the effect of these deletions on relative luciferase activity with or without 1 μM TO-901317 stimulation in mLXRα and mRXRα co-expressed OK cells. (B) Putative LXRE in the human FGF21 promoter. (C) An electrophoretic mobility shift assay was performed with or without mLXRα, mRXRα and 1 μM TO-901317 using the putative LXRE (−37 to 21 bp) in the human FGF21 promoter and a competitor (CYP7a1; −70 to −55 bp). Data represent mean±S.E. (n=3). *P<.05 as compared to DMSO.

regulation of transcriptional activity [16]. In the present study, we showed that LXR-mediated repression of FGF21 gene transcription and LXRα/RXRα binding to the human FGF21 promoter was increased by LXR ligand stimulation. Although the human FGF21 promoter contains a putative PPAR response element in the −696 to −685 region [17], no significant change was observed in LXR-mediated suppression of FGF21 promoter activity in a series of deletion mutants, including pFGF21-555, pFGF21-289 and pFGF21-83 mutant vectors. Moreover, PPARα agonist did not affect the promoter activity of the deletion mutants pFGF21-555 and pFGF21-289 (data not shown). These data suggest that LXR agonist-specific repressive effects are mediated via direct binding of liganded LXR to the human FGF21 promoter.

Recently, Yasutake et al. reported that dietary cholesterol is one of the important nutritional factors that affect nonalcoholic fatty liver disease (NAFLD) in nonobese patients [18]. As shown in this study, other researchers also showed that mice fed a cholesterol diet demonstrated fatty liver disease independent of BW [3]. In this condition, hepatic FGF21 and HSL gene expression was reduced in mice. We also showed that each mouse and human FGF21 gene expression was similarly suppressed by LXR stimulation. Thus, FGF21 may well be a useful target for drug development in the treatment of nonobese NAFLD patient as well as obese NAFLD patients. In fact, FGF21 already exert its anti-fatty liver effects in several mice models of NAFLD and metabolic syndrome [10,12].

In conclusion, we have shown that the human and mouse FGF21 gene is regulated by LXR in the liver, HepG2 cells and mouse primary hepatocytes. This regulation would protect hepatocytes from toxic cholesterol and/or its metabolites by reducing the supply of substrate from TG degradation to cholesterol biosynthesis. In other words, TG lipolysis in the liver may closely interact with cholesterol metabolism, and FGF21 may be a useful approach to treat fatty liver induced by excess cholesterol feeding.

References

[1] Kalaany NY, Gauthier KC, Zavacki AM, et al. LXRs regulate the balance between fat storage and oxidation. Cell Metab 2005;1:231–44.
[2] Makishima M. Nuclear receptors as targets for drug development: regulation of cholesterol and bile acid metabolism by nuclear receptors. J Pharmacol Sci 2005;97:177–83.
[3] Peet DJ, Turley SD, Ma W, et al. Cholesterol and bile acid metabolism are impaired in mice lacking the nuclear oxysterol receptor LXR alpha. Cell 1998;93:693–704.
[4] Sinal CJ, Tohkin M, Miyata M, Ward JM, Lambert G, Gonzalez FJ. Targeted disruption of the nuclear receptor FXR/BAR impairs bile acid and lipid homeostasis. Cell 2000;102:731–44.
[5] Apfel R, Benbrook D, Lernhardt E, Ortiz MA, Salbert G, Pfahl M. A novel orphan receptor specific for a subset of thyroid hormone-responsive elements and its interaction with the retinoid/thyroid hormone receptor subfamily. Mol Cell Biol 1994;14:7025–35.
[6] Song C, Kokontis JM, Hiipakka RA, Liao S. Ubiquitous receptor: a receptor that modulates gene activation by retinoic acid and thyroid hormone receptors. Proc Natl Acad Sci U S A 1994;91:10809–13.
[7] Wan YJ, An D, Cai Y, et al. Hepatocyte-specific mutation establishes retinoid X receptor alpha as a heterodimeric integrator of multiple physiological processes in the liver. Mol Cell Biol 2000;20:4436–44.
[8] Willy PJ, Umesono K, Ong ES, Evans RM, Heyman RA, Mangelsdorf DJ. LXR, a nuclear receptor that defines a distinct retinoid response pathway. Genes Dev 1995;9:1033–45.
[9] Badman MK, Pissios P, Kennedy AR, Koukos G, Flier JS, Maratos-Flier E. Hepatic fibroblast growth factor 21 is regulated by PPARalpha and is a key mediator of hepatic lipid metabolism in ketotic states. Cell Metab 2007;5:426–37.
[10] Coskun T, Bina HA, Schneider MA, et al. Fibroblast growth factor 21 corrects obesity in mice. Endocrinology 2008;149:6018–27.
[11] Inagaki T, Dutchak P, Zhao G, et al. Endocrine regulation of the fasting response by PPARalpha-mediated induction of fibroblast growth factor 21. Cell Metab 2007;5:415–25.
[12] Xu J, Lloyd DJ, Hale C, et al. Fibroblast growth factor 21 reverses hepatic steatosis, increases energy expenditure, and improves insulin sensitivity in diet-induced obese mice. Diabetes 2009;58:250–9.
[13] Uebanso T, Taketani Y, Fukaya M, et al. Hypocaloric high-protein diet improves fatty liver and hypertriglyceridemia in sucrose-fed obese rats via two pathways. Am J Physiol Endocrinol Metab 2009;297:E76–84.
[14] Mizuha Y, Yamamoto H, Sato T, et al. Water extract of *Cordyceps sinensis* (WECS) inhibits the RANKL-induced osteoclast differentiation. Biofactors 2007;30:105–16.
[15] Wagner BL, Valledor AF, Shao G, et al. Promoter-specific roles for liver X receptor/corepressor complexes in the regulation of ABCA1 and SREBP1 gene expression. Mol Cell Biol 2003;23:5780–9.
[16] Miyata KS, McCaw SE, Patel HV, Rachubinski RA, Capone JP. The orphan nuclear hormone receptor LXR alpha interacts with the peroxisome proliferator-activated receptor and inhibits peroxisome proliferator signaling. J Biol Chem 1996;271:9189–92.
[17] Lundasen T, Hunt MC, Nilsson LM, et al. PPARalpha is a key regulator of hepatic FGF21. Biochem Biophys Res Commun 2007;360:437–40.
[18] Yasutake K, Nakamuta M, Shima Y, et al. Nutritional investigation of non-obese patients with non-alcoholic fatty liver disease: the significance of dietary cholesterol. Scand J Gastroenterol 2009;44:471–7.