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RESEARCH ARTICLE

Liver X receptor α induces 17β-hydroxysteroid dehydrogenase-13 expression through SREBP-1c

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[1]*Center for Nephrology and Urology, Department of Physiology, Shenzhen University Health Science Center, Shenzhen University, Shenzhen, China;* [2]*AstraZeneca-Shenzhen University Joint Institute of Nephrology, Shenzhen University Health Science Center, Shenzhen, China;* [3]*Department of Physiology and Pathophysiology, Peking University Health Science Center, Beijing, China;* [4]*Center for Nuclear Receptors and Cell Signaling, Department of Biology and Biochemistry, University of Houston, Houston, Texas;* [5]*Advanced Institute for Medical Sciences, Dalian Medical University, Dalian, Liaoning, China; and* [6]*Center for Biosciences, Department of Biosciences and Nutrition, Karolinska Institutet, Novum, Stockholm, Sweden*

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Su W, Peng J, Li S, Dai Y, Wang C, Xu H, Gao M, Ruan X, Gustafsson J, Guan Y, Zhang X. Liver X receptor α induces 17β-hydroxysteroid dehydrogenase-13 expression through SREBP-1c. *Am J Physiol Endocrinol Metab* 312: E357–E367, 2017. First published March 7, 2017; doi:10.1152/ajpendo.00310.2016.—Liver X receptors, including LXRα and LXRβ, are known to be master regulators of liver lipid metabolism. Activation of LXRα increases hepatic lipid storage in lipid droplets (LDs). 17β-Hydroxysteroid dehydrogenase-13 (17β-HSD13), a recently identified liver-specific LD-associated protein, has been reported to be involved in the development of nonalcoholic fatty liver disease. However, little is known about its transcriptional regulation. In the present study, we aimed at determining whether 17β-HSD13 gene transcription is controlled by LXRs. We found that treatment with T0901317, a nonspecific LXR agonist, increased both 17β-HSD13 mRNA and protein levels in cultured hepatocytes. It also significantly upregulated hepatic 17β-HSD13 expression in wild-type (WT) and LXRβ$^{-/-}$ mice but not in LXRα$^{-/-}$ mice. Basal expression of 17β-HSD13 in the livers of LXRα$^{-/-}$ mice was lower than that in the livers of WT and LXRβ$^{-/-}$ mice. Moreover, induction of hepatic 17β-HSD13 expression by T0901317 was almost completely abolished in SREBP-1c$^{-/-}$ mice. Bioinformatics analysis revealed a consensus sterol regulatory element (SRE)-binding site in the promoter region of the 17β-HSD13 gene. A 17β-HSD13 gene promoter-driven luciferase reporter and ChIP assays further confirmed that the 17β-HSD13 gene was under direct control of SREBP-1c. Collectively, these findings demonstrate that LXRα activation induces 17β-HSD13 expression in a SREBP-1c-dependent manner. 17β-HSD13 may be involved in the development of LXRα-mediated fatty liver.

nonalcoholic fatty liver disease; SCDR9; lipid droplet; nuclear receptor; gene knockout; 17β-HSD13

NONALCOHOLIC FATTY LIVER DISEASE (NAFLD), which affects ~30% of adults in Western countries and 25% of subjects in the developing countries, has become a burgeoning health problem worldwide (4, 24, 34). Histologically, hepatic steatosis is characterized by a massive accumulation of lipid droplets (LDs) in more than 5% of hepatocytes, leading to inflammation and metabolic disturbances. NAFLD is one of the many consequences of chronic overnutrition and obesity, which encompasses a spectrum of liver diseases, including hepatic steatosis, steatohepatitis, cirrhosis, and hepatocellular carcinoma (26). To date, there is still a lack of an effective method for the diagnosis, staging, and treatment of NAFLD. Moreover, the pathogenesis of NAFLD remains poorly understood.

Liver X receptors (LXRs), including LXRα (NR1H3) and LXRβ (NR1H2), are members of the nuclear receptor (NR) superfamily. LXRα is expressed abundantly in tissues involved in active lipid metabolism, including the liver, adipose tissue, kidney, and macrophages, whereas LXRβ is expressed ubiquitously with high levels in the developing brain (5, 9). LXRs can be activated by naturally produced oxysterols and synthetic compounds such as T0901317 (10). They are key regulators in both cholesterol and fatty acid metabolism. In contrast to their role in atheroprotective cholesterol elimination (22), LXRs promote de novo lipogenesis. It has been reported that T0901317 induces hepatic lipogenesis via activating LXRs (28). The enhanced hepatic lipogenesis has been attributed mainly to the LXRα-dependent upregulation of sterol regulatory element-binding protein-1c (SREBP-1c) expression. LXRα and SREBP-1c have been described as major contributors to increased de novo lipogenesis in NAFLD (6, 13). LXRs bind directly to a consensus recognition sequence in the promoter region of the SREBP-1c gene and upregulate SREBP-1c expression, leading to enhanced fatty acid and triglyceride synthesis.

17β-Hydroxysteroid dehydrogenase 13 (17β-HSD13), originally named SCDR9, was cloned from a human liver cDNA library in 2007 (15). It belongs to the family of 17β-HSDs, which consists of 14 enzymes responsible for reduction or oxidation of sex hormones, fatty acids, and bile acids in vivo. The majority of the members of the family have a role in sex steroid metabolism, whereas a few 17β-HSD enzymes play key roles in cholesterol biosynthesis and elongation and oxidation of fatty acids (17, 25, 27). We found that, as a new member of this family, 17β-HSD13 was located on the surface of lipid droplets in the livers of patients and mice with NAFLD (7, 30), suggesting this enzyme may play an important role in the pathogenesis of fatty liver.

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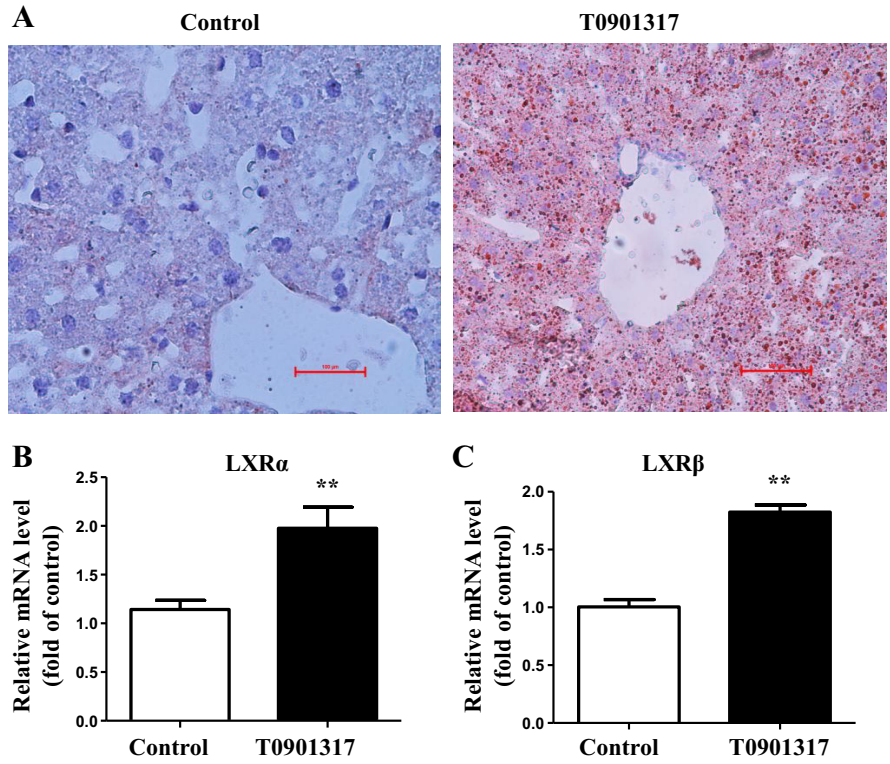

Fig. 1. Induction of liver steatosis by the liver X receptor (LXR) agonist T0901317 in mouse liver. *A*: Oil Red O staining showing an excessive lipid accumulation in C57Bl/6 mouse livers after treatment with T0901317 for 5 days. *B* and *C*: quantitative PCR analysis demonstrating LXRα (*B*) and LXRβ (*C*) mRNA levels, which were increased significantly after T0901317 treatment. Values are presented as means ± SE. $^{**}P < 0.01$ vs. control (untreated) group; $n = 3$. Scale bar, 100 μm.

Many LD proteins are regulated by the LXR/SREBP-1c pathway, such as CIDEA and PNPLA3 (14). These findings suggest that LXR might regulate LD formation through the regulation of LD-associated proteins. Gene profiling analysis revealed that LXR activation by T0901317 may also regulate gene expression of several members of the 17β-HSD family, such as 17β-HSD2, 17β-HSD5, and 17β-HSD11 (29, 31), yet the mechanism is essentially unknown. Here, we hypothesized that 17β-HSD13 may also be under the control of LXRs. In the present study, we provide evidence that T0901317 treatment induces 17β-HSD13 expression in mouse liver and cultured hepatocytes. This effect was mediated by LXRα in an SREBP-1c-dependent manner. Our findings may help clarify the underlying mechanisms by which LXRs modulate hepatic lipid metabolism through regulating the function of LD-associated proteins.

MATERIALS AND METHODS

Chemical reagents. T0901317 was purchased from Cayman Chemical (Ann Arbor, MI). TRIzol was purchased from Invitrogen (Carlsbad, CA). Reverse transcription and probe-labeling kits were purchased from Promega (Madison, WI). Quantitative real-time polymerase chain reaction (RT-PCR) was performed using a PTC-200 (MJ Research, St. Bruno, QC, Canada), with reagents obtained from BioTeke (Beijing, China). Antibodies (Abs) against SREBP-1c (2A4), FAS (H-300), and eukaryotic initiation factor 5 (C-14) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA), and 17β-HSD13 antibody was obtained from Abcam (ab122036). Horseradish-peroxidase-coupled secondary antibodies were purchased from Zhongshan Golden Bridge (ZSGB-Bio). Transient transfection reagent (Lipofectamine 2000) was purchased from Invitrogen.

Animals and treatment. All experiments were reviewed and approved by the Animal Care and Use Review Committee of Shenzhen

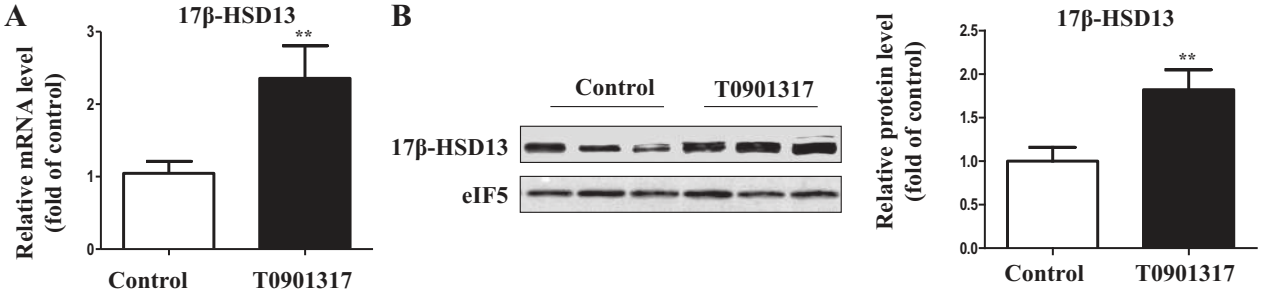

Fig. 2. Induction of 17β-hydroxysteroid dehydrogenase-13 (17β-HSD13) expression by LXR agonist T0901317 in mouse liver. *A*: real-time PCR assay demonstrating a significant increase in 17β-HSD13 mRNA levels in the livers of C57/BL6 mice treated with T0901317. *B*: Western blot analysis showing T0901317-induced 17β-HSD13 protein expression in mouse livers. Quantitation of 17β-HSD13 protein levels was presented. Values are presented as means ± SE. $^{**}P < 0.01$ vs. control group; $n = 3$. eIF5, eukaryotic initiation factor 5.

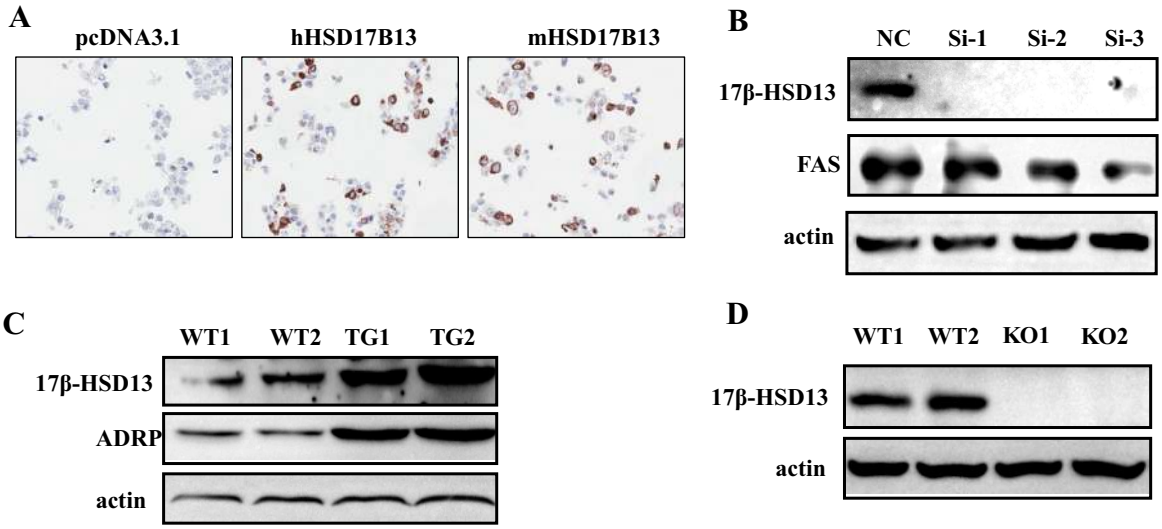

Fig. 3. Validation of 17β-HSD13 antibody. *A*: immunohistochemistry showed that little 17β-HSD13 immunoreactivity was detected by the antibody (ab122036) in human embryonic kidney-293 cells. However, after transfection of the cells with human and mouse 17β-HSD13 plasmids for 24 h, ab122036 could detect strong signals of both human and mouse 17β-HSD13 protein. *B*: in vitro validation of ab122036 in the HepG2 cell line. Three different siRNAs targeting 17β-HSD13 mRNA were transfected for 72 h, and the expression levels of 17β-HSD13 were determined using the antibody ab122036. Compared with control cells (NC), 17β-HSD13 siRNA-treated cells (si-1, si-2, and si-3) showed little 17β-HSD13 protein expression. *C*: Western blot assay showed that 17β-HSD13 and adipose differentiation-related protein (ADRP) expression were markedly induced in the livers of liver-specific 17β-HSD13 transgenic mouse lines (TG1 and TG2). WT1 and WT2, wild-type mouse lines. *D*: Western blot analysis showed that no positive signal was detected in 17β-HSD13-knockout (KO) mice. FAS, fatty acid synthase.

University. Male C57Bl/6 mice (8 wk old) were purchased from the Institute of Medical Laboratory Animal Center, Guangdong, China. LXRα- and LXRβ-null mice were generated by Dr. J. A. Gustafsson (Karolinska Institute, Huddinge, Sweden). SREBP-1c-null mice were purchased from The Jackson Laboratory (stock no. 004365). All animals were maintained on standard mouse chow and housed on a 12-h light-dark cycle under controlled temperature (22–24°C) and humidity (50−65%) in the facility at Shenzhen University Health Science Center. At 10 wk of age, animals were treated with LXR agonist T0901317 (5 $mg \cdot kg^{-1} \cdot day^{-1}$) or vehicle alone by gavage for 5 days, as reported previously (36, 38). At the end of the study, tissue samples were collected and snap-frozen in liquid nitrogen and then stored at −80°C until being processed further. Liver samples were also fixed in 4% paraformaldehyde solution for immunostaining.

Western blot analysis. Cell lysates were centrifuged at 12,000 *g* at 4°C for 15 min, and the supernatants were kept for Western blot analysis. Protein concentration was determined using the Pierce BCA kit. To determine expression levels of selected proteins, 60 μg of liver protein was separated by 10% SDS-polyacrylamide gel electrophoresis (PAGE). After incubation with a rabbit anti-17β-HSD13 antibody at 1:1,500 dilution, the blots were washed and incubated with peroxidase-conjugated secondary antibodies. After being washed three times, the membrane was then transferred to the ECL Reagent (Bio-Rad) and exposed to Imagequant LAS4000.

Primary culture of mouse hepatocytes. Primary cultured mouse hepatocytes were prepared as reported previously (20, 32). Briefly, mice were anesthetized with an intraperitoneal injection of bromethol. Livers were subjected to two-step collagenase perfusion through the portal vein. After the two-step perfusion, livers were excised and hepatocytes lushed out and filtered through a 220-μm pore size mesh nylon filter. Cells were washed three times. Cell viability was 85–95%, as evaluated by trypan blue exclusion. Hepatocytes were seeded in rat tail collagen-coated plates and cultured with RPMI 1640 medium containing 10% (vol/vol) FBS (fetal bovine serum), 2.5 nM insulin, 1 nM dexamethasone, 50 U/ml penicillin, and 50 μg/ml streptomycin. After 2 h, unattached cells were washed away with PBS, and fresh medium was added for an overnight incubation.

Oil Red O staining. Briefly, liver frozen section slides or cultured cells after T0901317 treatment were fixed in formaldehyde solution for 15 min. After being washed in distilled water, samples were immersed in 60% isopropanol for 1 min and then stained in filtered Oil Red O isopropanol solution for 10 min. After washing, samples were counterstained with hematoxylin for nuclei, visualized under light microscopy, and photographed.

Transfection and luciferase assay. Expression vectors encoding human SREBP-1c (N) were obtained from Prof. Y. Chang (Chinese Academy of Medical Science and Peking Union Medical College). HepG2 cells were transfected with a wild-type human 17β-HSD13 gene promoter-driven luciferase reporter (PV17B13) or SRE-mutant promoter-driven luciferase reporter (PMG86A) with or without a constitutively active form of SREBP-1c expression vector using lipotetamin 2000 (Invitrogen). After 48 h, cells were harvested in 1× luciferase lysis buffer (dual luciferase kit; Promega). Luciferase ac-

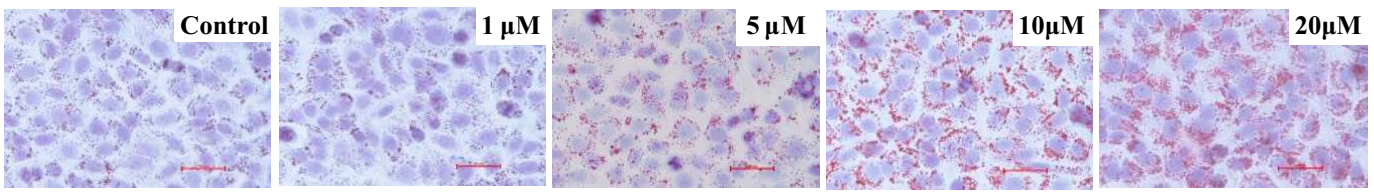

Fig. 4. T0901317 treatment induces lipid droplet formation in cultured hepatocytes. HepG2 cells were treated with different concentrations of T0901317 for 36 h. Oil Red O staining showed that T0901317 treatment induces a dose-dependent increase of lipid accumulation in cultured HepG2 cells. Scale bar, 100 μm.

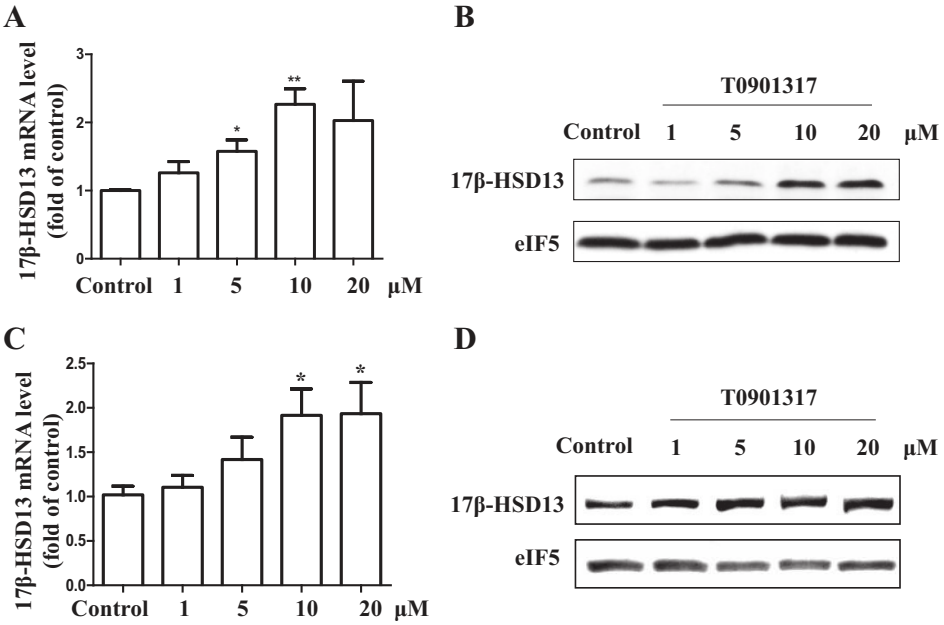

Fig. 5. T0901317 treatment increases 17β-HSD13 expression in cultured hepatocytes. *A*: real-time PCR assay demonstrating that treatment of the human hepatocarcinoma cell line HepG2 with T0901317 increased 17β-HSD13 mRNA expression in a dose-dependent manner. *B*: Western blot analysis showing that T0901317 induced 17β-HSD13 protein expression in a dose-dependent manner in HepG2 cells. *C*: T0901317 treatment induced 17β-HSD13 mRNA expression in primary cultured mouse hepatocytes in a dose-dependent manner; *D*: T0901317 treatment dose-dependently increased 17β-HSD13 protein levels in primary cultured mouse hepatocytes. Values are presented as means ± SE and obtained from 3 individual experiments. $*P < 0.05$ and $**P < 0.01$ vs. control group.

tivity was normalized to the β-galactosidase activity as measured by a kit.

Chromatin immunoprecipitation assay. Chromatin immunoprecipitation (ChIP) assay was carried out by using the Chromatin Immunoprecipitation Kit (Thermo). HepG2 cells were treated with T0901317 or DMSO for 24 h, and ChIP assay was performed. 3×10^5 cells were used for each ChIP assay. Anti-SREBP-1 antibody and normal mouse IgG (negative control) were used for immunoprecipitation of protein-DNA complexes. Precipitated DNA was analyzed by PCR using the following primers: 5-TATTTGAGGTGTTTCAAACC-3 (sense) and 5-TCACAGAGCTCTAGGGAAGG-3 (antisense). Amplification products were then examined by electrophoresis.

Statistical analysis. Statistical analysis was performed with GraphPad Prism version 5. Results were presented as means ± SE. Statistical analysis was performed using one-way ANOVA analysis of variance, followed by *t*-test. A value of $P < 0.05$ was considered statistically significant.

RESULTS

17β-HSD13 expression is upregulated in the livers of mice treated with T0901317. To determine the effect of LXR activation on 17β-HSD13 expression in mouse liver, we treated C57BL/6 mice with T0901317, a nonselective LXR agonist, (5

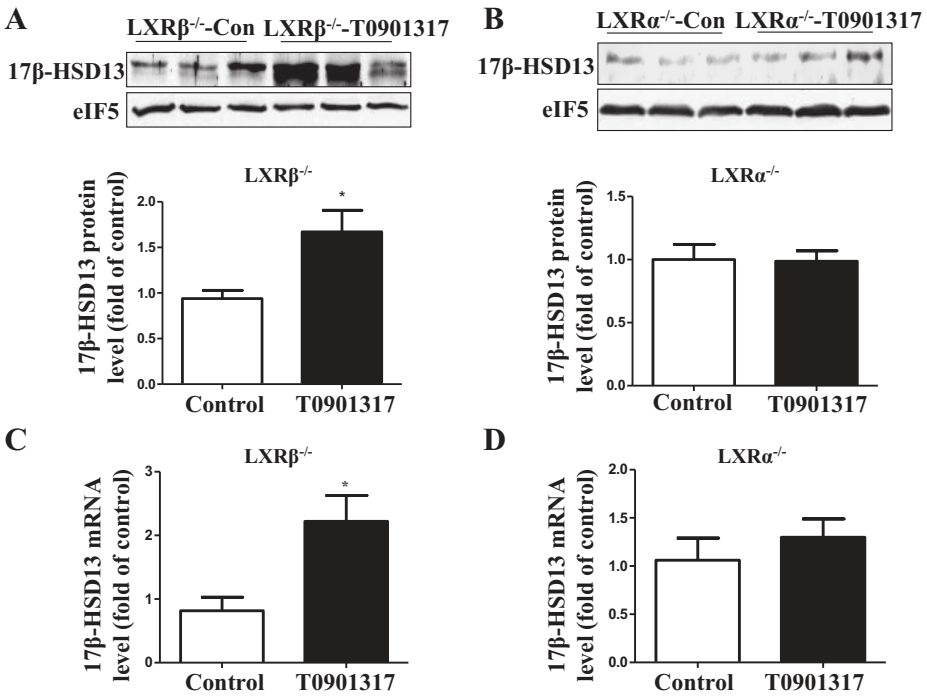

Fig. 6. T0901317-induced 17β-HSD13 expression in mouse liver is mediated by LXRα but not LXRβ. *A*: Western blot analysis showing that T0901317 treatment significantly induced 17β-HSD13 protein expression in livers of LXRβ$^{-/-}$ mice. *B*: immunoblotting analysis showing that T0901317 treatment failed to induce 17β-HSD13 protein expression in livers of LXRα$^{-/-}$mice. Quantification is shown below. *C*: quantitative PCR analysis demonstrating a significant induction of 17β-HSD13 mRNA expression by T0901317 in LXRβ$^{-/-}$ mice. *D*: real-time PCR assay demonstrating that T0901317 treatment had little effect on 17β-HSD13 mRNA levels in LXRα$^{-/-}$ mouse livers. Values are presented as means ± SE. $*P < 0.05$ vs. LXRβ$^{-/-}$-control (Con); $n = 4$.

mg·kg^{-1}·day^{-1} via gavage) for 5 days. As reported previously (33, 36), mice treated with T0901317 exhibited a significant fatty liver phenotype with increased liver triglyceride (TG) contents (Fig. 1*A*). T0901317 treatment also significantly increased the mRNA levels of LXRα and LXRβ (Fig. 1, *B* and *C*). Hepatic 17β-HSD13 mRNA levels were increased 2.35-fold in T0901317-treated mice compared with control (Fig. 2*A*). Consistently, Western blot assay revealed that T0901317 treatment increased the protein levels of 17β-HSD13 approximately twofold in the livers (Fig. 2*B*). These findings suggest that activation of LXRα and/or LXRβ increases hepatic 17β-HSD13 expression in vivo. The specificity of 17β-HSD13 antibody was validated both in vitro (Fig. 3, *A* and *B*) and in vivo (Fig. 3, *C* and *D*).

17β-HSD13 expression is induced in T0901317-treated cultured hepatocytes. We next determined the effect of T0901317 on 17β-HSD13 expression in a human hepatoma cell line. HepG2 cells were treated with different concentrations of T0901317. Oil Red O staining indicated that T0901317 treatment induced LD formation in HepG2 cells (Fig. 4). T0901317 treatment also resulted in a dose-dependent induction of 17β-HSD13 expression at both mRNA and protein levels (Fig. 5, *A* and *B*). Similarly, in primary cultured mouse hepatocytes, T0901317 treatment also induced 17β-HSD13 expression in a dose-dependent manner (Fig. 5, *C* and *D*). Collectively, the data indicate that 17β-HSD13 expression is induced by the LXR agonist T0901317 in cultured human and mouse hepatocytes in vitro.

LXRα, but not LXRβ, mediates T0901317-induced 17β-HSD13 expression in mouse livers. T0901317 is a nonselective agonist for both LXRα and LXRβ. To clarify which LXR isoform mediates T0901317-induced 17β-HSD13 expression in the livers, we treated both LXRα gene knockout (LXRα$^{-/-}$) mice and LXRβ gene knockout (LXRβ$^{-/-}$) mice with T0901317 and measured 17β-HSD13 expression at both mRNA and protein levels. T0901317 treatment significantly increased 17β-HSD13 mRNA and protein expression in wild-type mice (Fig. 2) and in LXRβ$^{-/-}$ mice (Fig. 6, *A* and *C*) but failed to induce 17β-HSD13 expression in LXRα$^{-/-}$ mice both on mRNA and protein levels (Fig. 6, *B* and *D*). These findings suggest that the LXRα isoform, rather than the LXRβ

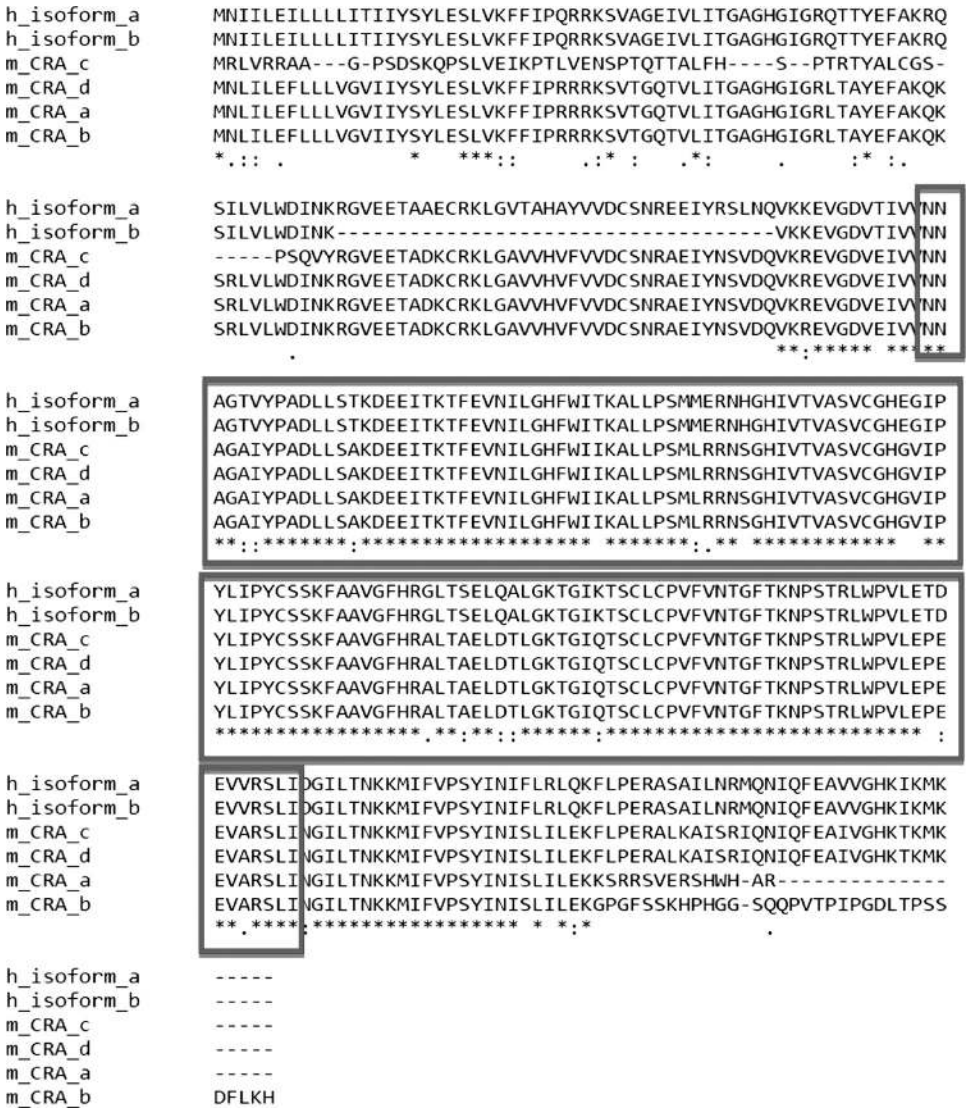

Fig. 7. Multiple sequence alignment of 2 human 17β-HSD13 isoforms and 4 mouse 17β-HSD13 isoforms. The peptide sequences used for generating the antibody ab122036 (amino acids 119–247 of human 17β-HSD13) are highlighted in gray boxes. *Identical amino acid; ⁻, scoring >0.5 in the Gonnet PAM 250 matrix; **:**, conservation between groups of strongly similar properties; period, conservation between groups of weakly similar properties. Sequences were analyzed using the ClustalW2 software.

isoform, is responsible for T0901317-induced 17β-HSD13 upregulation in the liver.

As shown in Fig. 6*A*, Western blot analysis revealed two bands of 17β-HSD13 that may be the result of alternative splicing of the mouse 17β-HSD13 gene. A search for the National Center for Biotechnology Information protein database found that there are four isoforms for mouse 17β-HSD13, including isoform CRA_a (GenBank accession no. EDL20236.1, 285aa), isoform CRA_b (GenBank accession no.EDL20237.1, 304aa), isoform CRA_c (GenBank accession no.EDL20238.1, 284aa), and isoform CRA_d (GenBank accession no. EDL20239.1, 300aa), with the maximal difference in molecular weight ~2 kDa (Fig. 7). It is also possible that the existence of two bands may be due to currently uncharacterized protein modification of 17β-HSD13.

Basal levels of 17β-HSD13 in the liver are determined by LXRα. To further confirm the role of LXRα in hepatic 17β-HSD13 expression, we examined the basal levels of 17β-HSD13 in the livers of wild-type, LXRα$^{-/-}$, and LXRβ$^{-/-}$ mice. As shown in Fig. 8, the basal levels of 17β-HSD13 expression were significantly lower in LXRα$^{-/-}$ mice than in wild-type mice (Fig. 8, *A*, *C*, and *E*). However, 17β-HSD13 expression levels were comparable between wild-type and LXRβ$^{-/-}$ mice (Fig. 8, *B*, *D*, and *E*). These findings further support that T0901317-induced 17β-HSD13 upregulation was mediated mainly by LXRα, but not LXRβ, in the liver.

As reported previously (22, 37), we found that LXRα$^{-/-}$ mice displayed a steatosis-like morphology in the liver (Fig. 8*E* and Fig. 9). Immunohistochemistry analysis showed that both 17β-HSD13 and adipose differentiation-related protein (ADRP), two well-documented LD-associated proteins, were located on the surface of intracellular lipid droplets (Figs. 8*E* and 9). However, unlike ADRP expression, which was slightly upregulated (Fig. 9), 17β-HSD13 expression was markedly attenuated in the livers of LXRα$^{-/-}$ mice (Fig. 8*E*). These findings suggest that LXRα has a distinct effect on the expression of 17β-HSD13 and ADRP, two abundant hepatic LD-associated proteins. It also suggests that 17β-HSD13 might participate in LXRα-mediated hepatic steatosis.

Transcriptional regulation of 17β-HSD13 by LXRα is SREBP-1c dependent. To determine the molecular mechanisms by which LXRα induces 17β-HSD13 expression in the liver, we analyzed the promoter region of the human 17β-HSD13 gene by using the TESS (http://www.cbil.upenn.edu/cgi-bin/tess/tess). No potential LXR response elements (LXREs) were found. Nevertheless, a putative steroid regulatory element (SRE) was identified. Since SREBP-1c is a direct target gene of LXRs that mediates most of the lipogenic effects of LXRs (8), we hypothesized that 17β-HSD13 may be regulated by LXRα via SREBP-1c. As expected, SREBP-1c mRNA levels were absent in SREBP-1c$^{-/-}$ mice and significantly upregulated in the livers of wild-type mice receiving T0901317

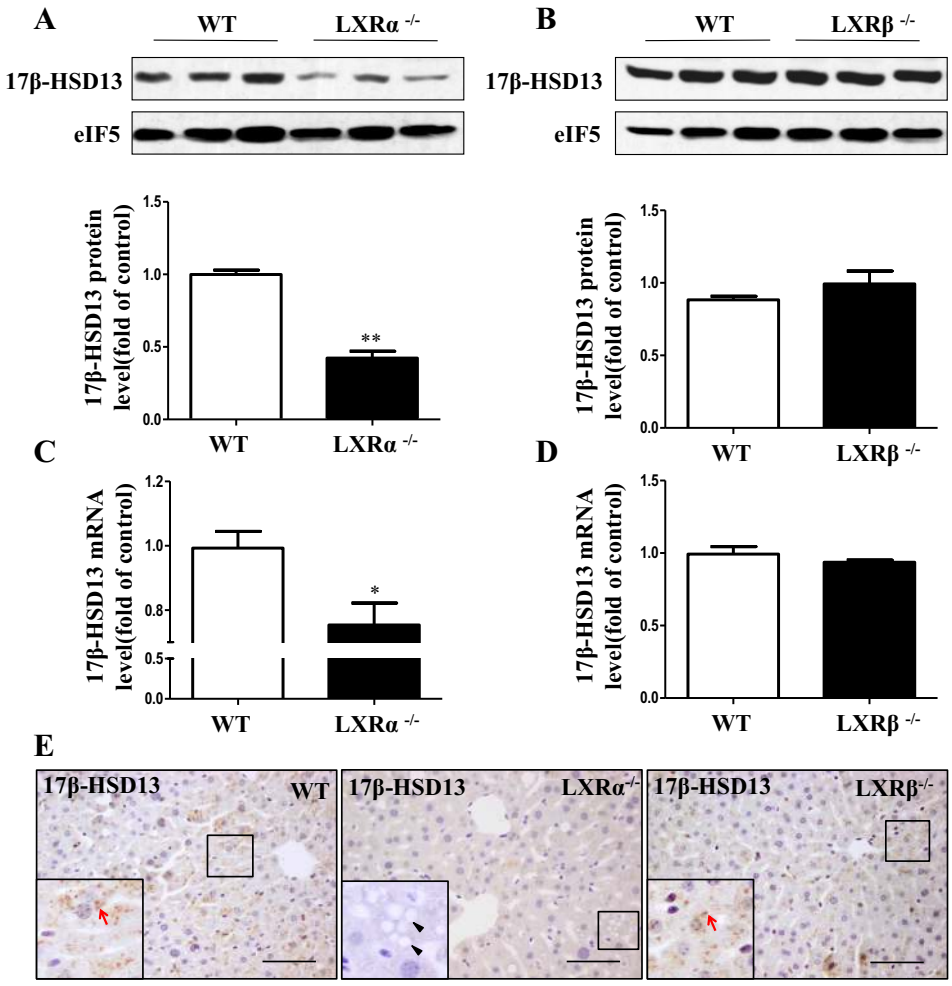

Fig. 8. Basal 17β-HSD13 expression levels are reduced in the livers of LXRα$^{-/-}$ mice but not in LXRβ$^{-/-}$ mice. *A*: immunoblot assay showing that hepatic 17β-HSD13 protein expression was significantly reduced in LXRα$^{-/-}$ mice. **$P < 0.01$ vs. wild type (WT); $n = 3$. *B*: Western blot assay demonstrating no difference in hepatic 17β-HSD13 protein levels between WT and LXRβ$^{-/-}$ mice. Quantitative measurements of 17β-HSD13 protein levels were performed. *C*: real-time PCR assay also showed that 17β-HSD13 mRNA level was reduced in LXRα$^{-/-}$ mice. *$P < 0.05$ vs. WT; $n = 3$. *D*: no difference in hepatic 17β-HSD13 mRNA levels between WT and LXRβ$^{-/-}$ mice. *E*: immunohistochemistry showing that 17β-HSD13 was located on the surface of liver lipid droplets (LDs) of both WT and LXRβ$^{-/-}$ mice but was barely detectable in most of the LDs in LXRα$^{-/-}$ mice. LXRα$^{-/-}$ mice displayed steatotic morphology in the liver. Red arrows point to positive 17β-HSD13 immunoreactivity on the surface of LDs; black arrowheads point to large LDs in LXRα$^{-/-}$ mouse liver, showing very weak 17β-HSD13 immunostaining signal. Scale bar, 50 μm.

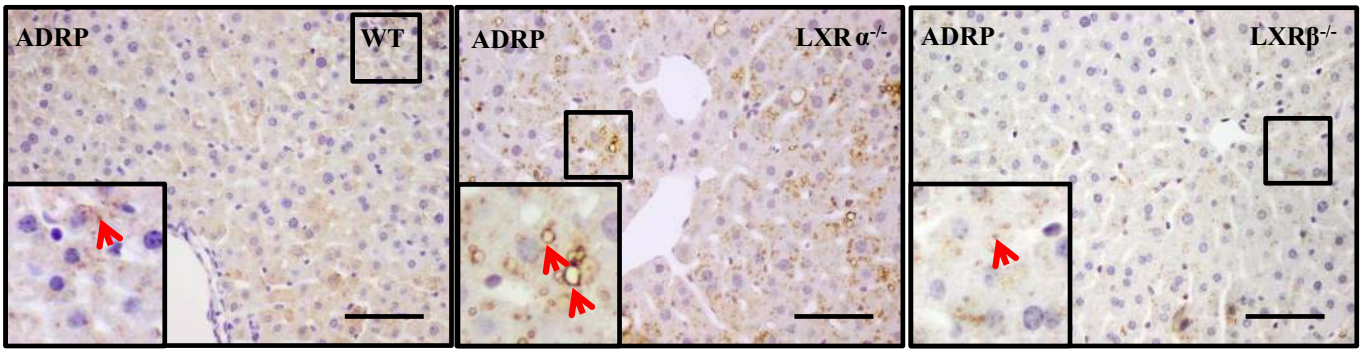

Fig. 9. Immunohistochemistry analysis of ADRP in WT, LXRα$^{-/-}$, and LXRβ$^{-/-}$ mice. LXRα$^{-/-}$ mice displayed steatotic morphology in the livers. Immunostaining showing that ADRP was localized mainly on the surface of liver lipid droplets (LD) of wild-type, LXRα$^{-/-}$, and LXRβ$^{-/-}$ mice. Note: LXRα$^{-/-}$ mice exhibited much stronger ADRP immunoreactivity than wild-type and LXRβ$^{-/-}$ mice. Red arrows point to positive ADRP immunoreactivity on the surface of LD. Scale bar, 50 μm.

treatment (Fig. 10*A*). LXR activation markedly increased hepatic 17β-HSD13 expression at both the mRNA and protein level in wild-type mice (Fig. 10, *B–D*). Notably, induction of hepatic 17β-HSD13 expression by the LXR agonist T0901317 was completely abolished in SREBP-1c$^{-/-}$ mice(Fig. 10, *B–D*). These findings demonstrate that LXRα-mediated 17β-HSD13 expression is SREBP-1c dependent.

17β-HSD13 is a direct target gene of SREBP-1c. To further characterize whether 17β-HSD13 is a direct target gene of SREBP-1c, ChIP and luciferase reporter assays were performed. HepG2 cells were treated with or without T0901317 for 24 h, after which ChIP assay was performed. As shown in Fig. 11*A*, the ChIP assay revealed a single band at the estimated size, indicating SREBP-1 can bind directly to the SRE region of the 17β-HSD13 gene promoter (Fig. 11*A*). Additionally, T0901317 treatment significantly enhanced the binding of SREBP-1 to 17β-HSD13 gene promoter (Fig. 11*B*). For the luciferase reporter assay, we amplified an ~1-kb human 17β-HSD13 gene promoter via PCR and then cloned it into the pGL3-basic vector, resulting in a human 17β-HSD13 gene promoter-driven luciferase reporter (PV17B13). By replacing nucleotide G with A at the site of −86, we constructed another human 17β-HSD13 gene promoter-driven luciferase reporter with a mutant SRE (PMG86A) (Fig. 11*C*). The luciferase assay showed that overexpression of SREBP-1c in HepG2 cells for 48 h significantly induced wild-type 17β-HSD13 gene promoter activity (~4.35-fold change) but failed to increase SRE-mutant 17β-HSD13 gene promoter activity (Fig. 11*D*). These results indicate that 17β-HSD13 is a direct target gene of SREBP-1c and the SRE site, and its gene promoter is responsible for SREBP-1c-induced upregulation of 17β-HSD13 expression.

DISCUSSION

In our previous work, we demonstrated that overexpression of a newly identified liver-specific lipid droplet (LD)-associated protein, 17β-HSD13, both in vitro and in vivo resulted in

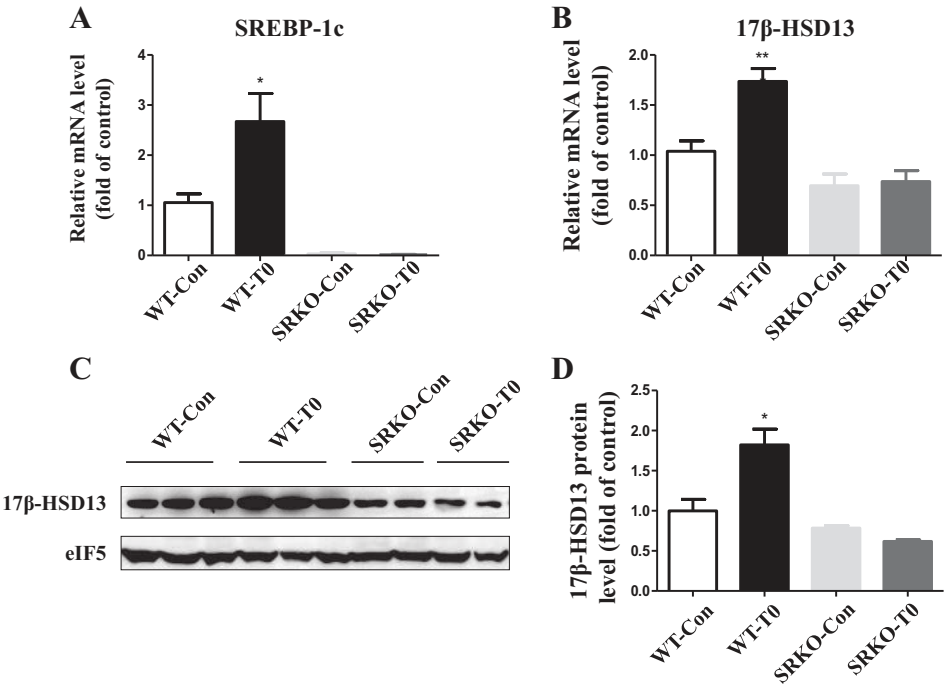

Fig. 10. Sterol regulatory element-binding protein-1c (SREBP-1c) is required for LXR agonist-induced hepatic 17β-HSD13 expression. *A*: quantitative PCR measurement demonstrating that LXR agonist T0901317 (T0) significantly induces SREBP-1c mRNA expression in wild-type (WT) mice but not in SREBP-1c$^{-/-}$ mice (SRKO). *$P < 0.05$ vs. WT-control (Con) (wild-type mice treated with DMSO); $n = 3$. *B*: real-time PCR assay showing that T0-induced hepatic 17β-HSD13 mRNA expression is completely abolished in SREBP-1c$^{-/-}$ mice. **$P < 0.01$ vs. WT-Con; $n = 3$. *C*: Western blot assay showing that T0-induced 17β-HSD13 protein expression is almost completely abolished in SREBP-1c$^{-/-}$ mice. Note: the basal levels of 17β-HSD13 expression are markedly reduced in SREBP-1c$^{-/-}$ mice compared with WT-Con mice. *D*: quantitative measurement of 17β-HSD13 protein levels as assessed by Western blotting shown in *C*. *$P < 0.05$ vs. WT-Con; $n = 3$.

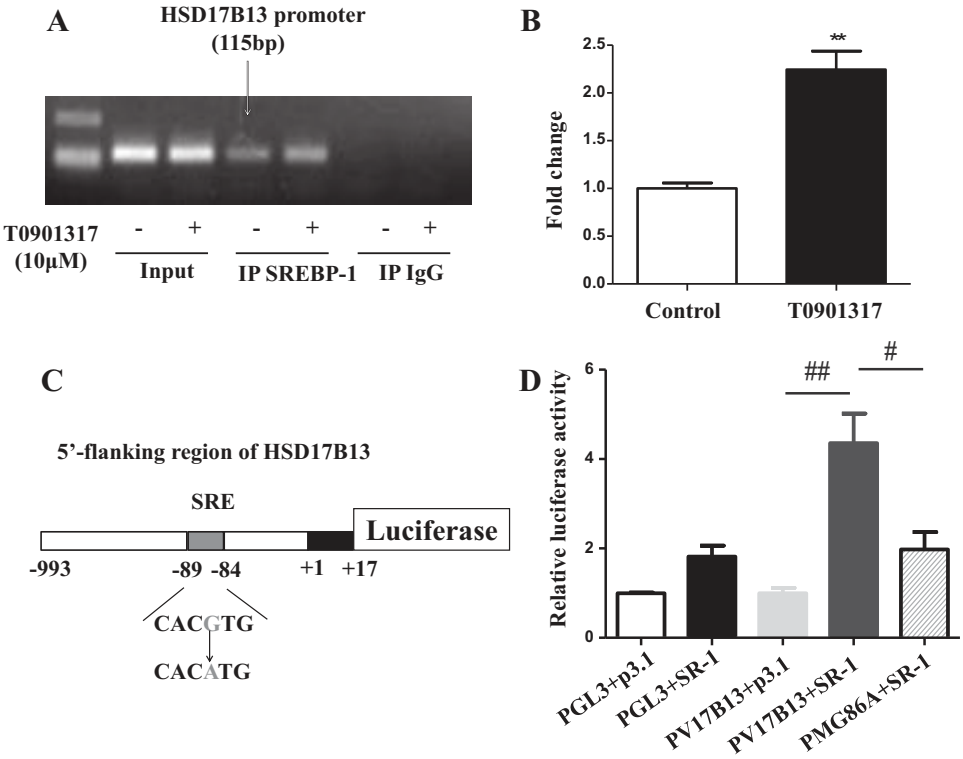

Fig. 11. 17β-HSD13 was transcriptionally regulated by SREBP-1c via binding to the SRE site. *A*: analysis of SREBP-1c binding to human 17β-HSD13 promoter by the chromatin immunoprecipitation (ChIP) assay. HepG2 cells were treated with or without T0901317 for 24 h before being harvested for the ChIP assay with indicated antibodies. Precipitated DNA was analyzed for protein occupancy at the indicated promoters by semiquantitative PCR. Note: a predicted PCR amplification band of 115 bp was evident. *B*: quantitative measurement of the ChIP products was performed. $^{**}P < 0.01$ vs. HepG2 cells untreated with T0901317 (control); $n = 3$. *C*: construction of human 17β-HSD13 gene promoter-driven luciferase reporter vectors; 1-kb human 17β-HSD13 gene promoter was amplified by PCR and then cloned into the pGL3-basic vector, resulting in a human 17β-HSD13 gene promoter-driven luciferase reporter (PV17B13). By replacing nucleotide G with A at −86 bp upstream of the transcription start site, another human 17β-HSD13 gene promoter-driven luciferase reporter with a mutant SRE was generated (PMG86A). *D*: overexpression of SREBP-1c induced transcriptional activity of the 17β-HSD13 gene promoter. PV17B13 or PMG86A reporter plasmids were transiently transfected with the pCDNA3.1 (p3.1) empty vector or the SREBP-1c-pCDNA3.1 expression vector (SR-1). After incubation for 48 h, transcriptional activity was determined by measuring the luciferase activity. Luciferase activity was normalized to β-galactosidase activity and presented as luciferase/β-galactosidase relative units. Values are presented as means ± SE. $\#P < 0.05$, PV17B13 + SR-1 vs. PMG86A + SR-1; $\#\#P < 0.01$, PV17B13 + SR-1 vs. PV17B13 + p3.1; $n = 3$.

significantly enhanced lipogenesis and an increase in the number and size of LDs. 17β-HSD13 may play a critical role in hepatic lipid homeostasis, and dysregulation of 17β-HSD13 may be involved in the pathogenesis of NAFLD (30). In the present study, we further unraveled the transcriptional regulation of the 17β-HSD13 gene. 17β-HSD13 expression was induced by the LXR agonist T0901317 in parallel with lipid accumulation in mouse livers and cultured hepatocytes. We also revealed for the first time that the induction of 17β-HSD13 in vivo by T0901317 was mediated by the activation of LXRα but not LXRβ. Furthermore, we provided evidence that LXRα-mediated hepatic 17β-HSD13 expression is SREBP-1c dependent. These findings demonstrate that 17β-HSD13 expression is under the control of LXRα in an SREBP-1c-dependent manner.

LXRs, including LXRα and LXRβ, play an important role in the regulation of cholesterol, lipid, and carbohydrate metabolism. Synthetic LXR agonists have been shown to improve insulin resistance (12) and display antiatherogenic properties due to their effects on reverse cholesterol transport mediated by increased cholesterol efflux (2). However, activation of LXRs also results in a significant fatty liver phenotype and hypertriglyceridemia due to the upregulation of hepatic lipogenic enzymes that trigger liver fatty acid and triglyceride synthesis (28). Although both isoforms share significant sequence similarity, LXRα and LXRβ have been found to exert overlapping but not identical functions in hepatic lipid metabolism (1, 3, 21). LXRα-null mice exhibit reduced hepatic expression of several genes involved in fatty acid synthesis (6), suggesting that SREBP-1c may mediate LXR agonist-induced hepatic lipid accumulation. In support, SREBP-1 mRNA is reduced in LXRα-null mice but not in mice lacking LXRβ gene (22). Furthermore, SREBP-1c is a direct target gene of LXRα but not LXRβ (23). More studies are needed to clarify the role of each LXR isoform in regulating hepatic lipid metabolism. Novel strategies are also required to separate the beneficial effects of LXR activation from the unwanted metabolic side effects.

The present study reveals for the first time that LXRα, but not LXRβ, is critical in controlling gene expression of 17β-HSD13 in the liver, suggesting that LXRα and LXRβ may play distinct roles in liver lipid metabolism. In vitro studies using primary cultured mouse hepatocytes from LXRα$^{-/-}$ and LXRβ$^{-/-}$ mice would be very helpful to clarify whether T0901317-induced 17β-HSD13 gene expression is LXRα dependent. Because of the lipogenic action of 17β-HSD13 in the

liver (30), induction of 17β-HSD13 expression may contribute to LXRα activation-associated hepatic steatosis.

LXRα and SREBP-1c have been described as major transcription factors contributing to increased de novo lipogenesis in NAFLD (6, 13). Our previous study demonstrates that expression of thyroid hormone-responsive SPOT 14 (THRSP), a lipogenic gene, is induced by the LXR agonist T0901317 in the liver through the LXRα-mediated, SREBP-1c dependent mechanism (11). Clinical studies have also revealed an important role of the LXRα/SREBP-1c pathway in the pathogenesis of NAFLD (6, 19). It has been reported previously that LXRα directly controls the expression of SREBP-1c by binding to a consensus recognition sequence in its promoter region, leading to enhanced fatty acid and triglyceride synthesis by activating the genes encoding acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), stearoyl CoA desaturase-1 (SCD-1), and THRSP (33). The present study demonstrated that 17β-HSD13 is also a direct target gene of SREBP-1c. A putative SRE sequence was found in the 5′-flanking region of the HSD17B13 gene. Furthermore, SREBP-1c can bind directly to the SRE region of the 17β-HSD13 promoter, inducing HSD17B13 gene promoter activity. SREBP-1c appears to be essential for basal 17β-HSD13 expression in the liver, consistent with the finding that basal levels of 17β-HSD13 expression were lower in SREBP-1c$^{-/-}$ mice than in wild-type mice. It was also found that LXRα-induced upregulation of 17β-HSD13 was markedly blunted in SREBP-1c$^{-/-}$ mice, suggesting that SREBP-1c is required for LXRα-mediated induction of 17β-HSD13 in mouse liver.

It has been reported previously that mice lacking LXRα lose the capacity to regulate the catabolism of dietary cholesterol in the liver, resulting in an accumulation of large amounts of cholesterol in liver (22, 37). Consistently, in the present study we also observed similar LD accumulation in LXRα$^{-/-}$ mouse livers. Interestingly, LXRα$^{-/-}$ mice exhibited a distinct expression pattern of LD-associated proteins in the livers. Hepatic expression levels of ADRP, one of the most abundant hepatic LD proteins, were even mildly increased in the livers of LXRα$^{-/-}$ mice compared with the livers of wild-type mice. However, hepatic expression levels of 17β-HSD13 in LXRα$^{-/-}$ mice were much lower than in wild-type mice. LDs in liver are heterogeneous in lipid/protein composition and function. Zhang et al. (35) recently successfully separated liver LDs into subpopulations relatively enriched in cholesterol ester (CE) or triacylglycerol (TAG), which provide the groundwork to search for a marker protein to differentiate these popula-

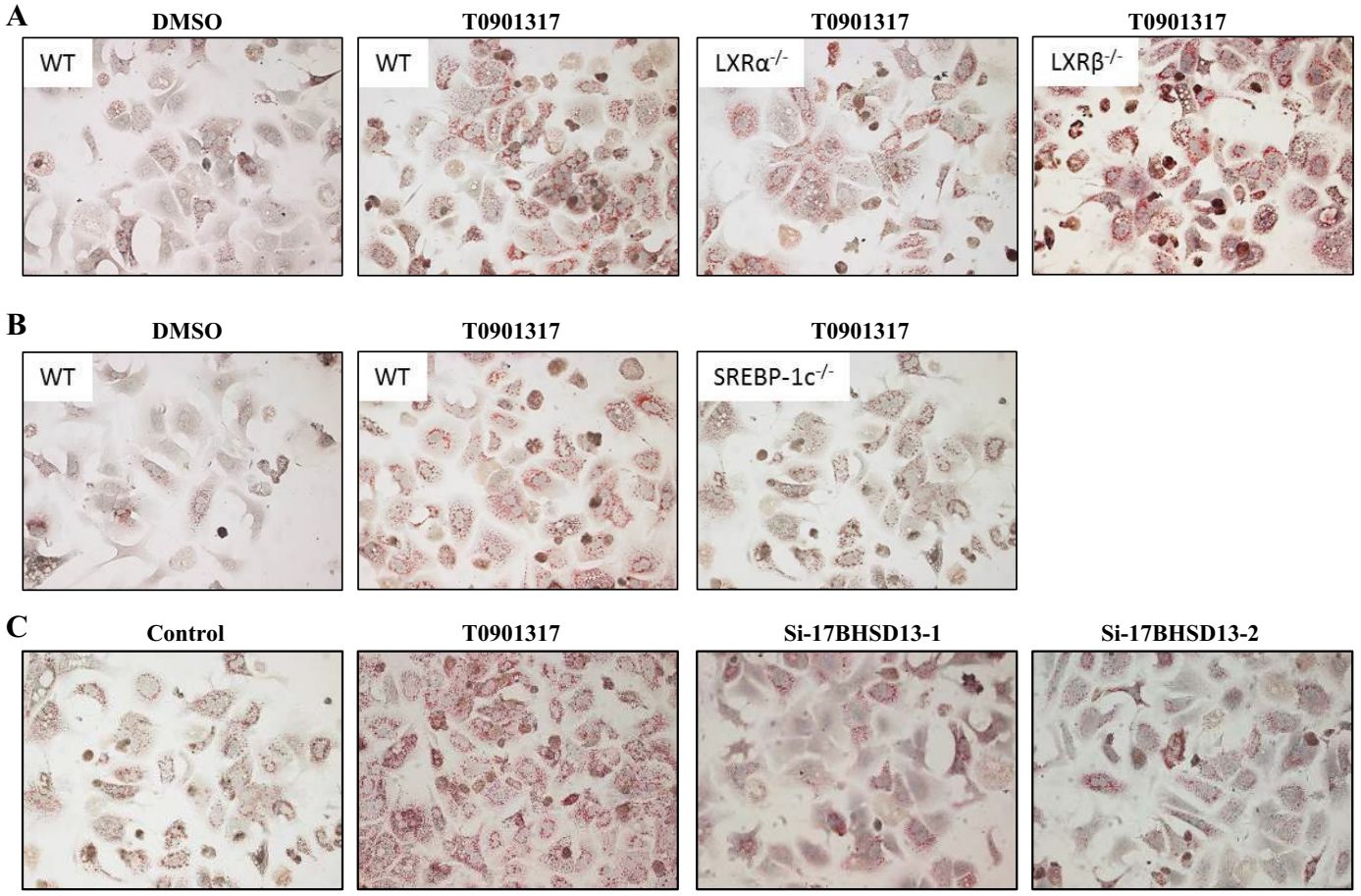

Fig. 12. The LXRα-SREBP-1c-17βHSD13 axis contributes to T0901317-induced liver cell steatosis in vitro. *A*: LXRα-mediated steatotic action of T0901317 in primary cultured mouse hepatocytes. Liver cells were cultured from wild-type (WT), LXRα$^{-/-}$, and LXRβ$^{-/-}$ mice on C57BL/6 background and treated with T0901317 for 36 h. Lipid accumulation was analyzed by Oil Red O staining. *B*: SREBP-1c-mediated steatotic action of T0901317 in primary cultured mouse hepatocytes. Liver cells were cultured from wild-type (WT) and SREBP-1c$^{-/-}$ mice on a 129Svj background and treated with T0901317 for 36 h. *C*: 17β-HSD13 mediating T0901317-elicited lipid accumulation in liver cells. Primary cultured hepatocytes from WT mice (on C57BL/6) were treated with T0901317 in the presence of 2 specific 17β-HSD13 siRNAs. Oil Red O staining was performed to visualize the lipid droplets in hepatocytes.

tions. The accumulated LDs in LXRα$^{-/-}$ mice might be CE enriched LDs but not TAG-enriched LDs, which is different from human NAFLD. One potential explanation for the low expression of 17β-HSD13 being associated with more LDs in the LXRα$^{-/-}$ livers might be that 17β-HSD13 is responsible mainly for hepatic steatosis related to enhanced fatty acid and triglyceride biosynthesis but not increased cholesterol accumulation. This is consistent with lower hepatic and plasma TG levels observed in LXRα$^{-/-}$ mice (37). It also suggests that 17β-HSD13 might participate in LXRα-mediated hepatic steatosis but not LXRα unrelated hepatic steatosis. These findings support our conclusion that 17β-HSD13 expression is regulated by LXRα. Our observations also demonstrate that as the two most abundant liver LD proteins, ADRP and 17β-HSD13 may be regulated by different mechanisms. It has been reported that both ADRP and 17β-HSD13 are upregulated in high-fat diet-induced hepatic steatosis (18, 30). However, ADRP is regulated by PPARγ (18), whereas 17β-HSD13 is under the control of LXRα. Therefore, multiple nuclear receptors and transcription factors may work in concert to precisely regulate hepatic lipid metabolism possibly through the regulation of gene expression and function of LD-associated proteins.

Although our in vivo results strongly support the LXRα/SREBP-1c pathway playing a critical role in hepatic 17β-HSD13 expression, we can't exclude other mechanisms involved in the effect of LXR agonist on hepatic lipid metabolism, such as THRSP and SCD1. Increasing evidence demonstrates that T0901317, the most commonly used LXR agonist, also represents a potent activator for other nuclear receptors such as PXR (16). In addition, the basal levels of hepatic 17β-HSD13 expression were only partially reduced in LXRα$^{-/-}$ mice, suggesting that other transcription factors are also involved in the regulation of 17β-HSD13 gene transcription. Our preliminary data demonstrate that primary cultured mouse hepatocytes with gene deficiency of LXRα and SREBP-1c, and gene knockdown of 17β-HSD13 exhibited a marked reduction in T0901317-induced lipid accumulation (Fig. 12). However, determining the effect of T0901317 on hepatic lipid metabolism in 17β-HSD13 gene-deficient mice would help address directly the question whether the effect of LXR agonist on hepatic steatosis is dependent on 17β-HSD13.

In conclusion, the present study demonstrates that hepatic 17β-HSD13 gene expression is induced by LXRα in an SREBP-1c-dependent manner. The LXRα/SREBP-1c/17β-HSD13 pathway may provide a therapeutic target for the development of novel drugs for the treatment of NAFLD.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

W.S., J.P., S.L., Y.-b.D., C.W., H.X., and M.G. performed experiments; W.S. and S.L. interpreted results of experiments; W.S. prepared figures; W.S. and J.P. drafted manuscript; W.S., J.P., C.W., X.-z.R., J.-A.G., X.-y.Z., and Y.-f.G. edited and revised manuscript; W.S., J.P., J.-A.G., X.-y.Z., and Y.-f.G. approved final version of manuscript; S.L., H.X., and M.G. analyzed data.

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