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Transcriptional Regulation of Human Hydroxysteroid Sulfotransferase SULT2A1 by LXRα

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ABSTRACT

The nuclear receptor liver X receptor (LXR) plays an important role in the metabolism and homeostasis of cholesterol, lipids, bile acids, and steroid hormones. In this study, we uncovered a function of LXRα (NR1H3) in regulating the human hydroxysteroid sulfotransferase SULT2A1, a phase II conjugating enzyme known to sulfonate bile acids, hydroxysteroid dehydroepiandrosterone, and related androgens. We showed that activation of LXR induced the expression of SULT2A1 at mRNA, protein, and enzymatic levels. A combination of promoter reporter gene and chromatin immunoprecipitation assays showed that LXRα transactivated the SULT2A1 gene promoter through its specific binding to the −500- to −258-base pair region of the SULT2A1 gene promoter. LXR small interfering RNA knockdown experiments suggested that LXRα, but not LXRβ, played a dominant role in regulating SULT2A1. In primary human hepatocytes, we found a positive correlation between the expression of SULT2A1 and LXRα, which further supported the regulation of SULT2A1 by LXRα. In summary, our results established human SULT2A1 as a novel LXRα target gene. The expression of LXRα is a potential predictor for the expression of SULT2A1 in human liver.

Introduction

Liver X receptor (LXR) belongs to the nuclear receptor superfamily of ligand-dependent transcription factors. LXR contains two isoforms, LXRα and LXRβ. LXRα shows enriched expression in the liver, kidney, intestine, lung, spleen, fat tissues, and macrophages, whereas LXRβ is expressed ubiquitously (Tontonoz and Mangelsdorf, 2003). When activated by its endogenous (Janowski et al., 1996; Forman et al., 1997; Lehmann et al., 1997; Song and Liao, 2000) or synthetic ligands (Schultz et al., 2000; Collins et al., 2002), LXR forms heterodimers with the retinoid X receptor, and then binds to LXR response elements in the promoters of LXR target genes.

In humans, LXR regulates a wide range of genes, including ATP-binding cassette transporter isoforms A1, G1, G5, and G8; apolipoprotein E; cholesteryl ester transfer protein; lipoprotein lipase; fatty acid synthase; and sterol regulatory element–binding protein-1c (Edwards et al., 2002). Through its gene regulatory activities, LXR is involved in diverse physiologic functions in rodents, including cholesterol and lipid metabolism (Repa et al., 2000; Schultz et al., 2000; Repa and Mangelsdorf, 2002), anti-inflammation (Joseph et al., 2004; Zelcer and Tontonoz, 2006), hepatobiliary disease (Uppal et al., 2007, 2008), acetaminophen liver toxicity (Saini et al., 2011), and steroid hormone biosynthesis and metabolism (Cummins et al., 2006; Gong et al., 2007; Lee et al., 2008).

Sulfotransferases (SULTs) are phase II drug-metabolizing enzymes that catalyze the sulfonation of endogenous and exogenous hydroxyl-containing compounds. SULT2A1, a member of the hydroxysteroid SULT subfamily that is highly expressed in the liver and adrenal gland (Falany, 1997), catalyzes the metabolism of bile acids (Radominska et al., 1990), hydroxysteroid dehydroepiandrosterone (Otterness et al., 1992), and related androgens. In rodents, Sult2a1-mediated bile acid sulfonation has been shown to play an important role in bile acid detoxification and prevention of cholestasis (Uppal et al., 2007). The expression of human or rodent SULT2A1 is transcriptionally regulated by several nuclear receptors, such as the pregnane X receptor (PXR) (Sonoda et al., 2002), constitutive androstane receptor (Saini et al., 2004), hepatocyte nuclear factor-4 (Fang et al., 2007), farnesoid X receptor (Song et al., 2001), estrogen-related receptor-α (Seely et al., 2005), and retinoic acid receptor–related orphan receptor (ROR) α and RORγ (Ou et al., 2013). In rodents, the expression of Sult2a1 was shown to be regulated by LXR, which was postulated to account for the anticholestatic activity of LXR (Uppal et al., 2007). However, the regulatory effect of LXR on human SULT2A1 gene expression remains unclear.

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ABBREVIATIONS: bp, base pairs; β-gal, β-galactosidase; GW3965, 3-[3-[[[2-chloro-3-(trifluoromethyl)phenyl](2,2-diphenylethyl)amino]propoxy] benzeneacetic acid hydrochloride; LXR, liver X receptor; PAPS, phosphoadenosine phosphosulfate; PCR, polymerase chain reaction; PXR, pregnane X receptor; ROR, retinoic acid receptor–related orphan receptor; siRNA, small interfering RNA; SULT, sulfotransferase; TO901317, *N*-(2,2,2-trifluoroethyl)-*N*-[4-[2,2,2-trifluoro-1-hydroxy-1-(trifluoromethyl)ethyl]phenyl]benzenesulfonamide.

In this study, we showed that LXRα positively and directly regulated SULT2A1 gene expression in human liver cells, suggesting that this regulation is conserved in rodents and humans. The regulatory effect of LXRα on SULT2A1 gene expression was further supported by the positive association between LXRα and SULT2A1 expression in a cohort of primary human hepatocytes.

Materials and Methods

Reagents and Chemicals. GW3965 [3-[3-[[[2-chloro-3-(trifluoromethyl) phenyl](2,2-diphenylethyl)amino]propoxy]benzeneacetic acid hydrochloride] was synthesized in-house as previously described (Collins et al., 2002). All other chemicals, if not specified, were purchased from Sigma-Aldrich (St. Louis, MO).

Plasmid Constructs. The pGL3-SULT2A1 promoter reporters that contain 1250 base pairs (bp) of the SULT2A1 gene promoter or its deletion mutants were cloned by polymerase chain reaction (PCR) using the following forward primers: 1250-bp, 5′-CGCGAGCTCGCCAACTGATCTGTTGTAT-3′; 850-bp, 5′-CGCGAGCTCTATGCAAACAAATCTTTCC-3′; 660-bp, 5′-CGCGAGCTCAGGTATAATTGTGTGATAC-3′; 500-bp, 5′-CGCGAGCTCTGAGAACAGATAAAGACTGT-3′; and 250-bp, 5′-CGCGAGCTCATCTTGCAGTTCACTCTCAG-3′. The common reverse primer was 5′-CCGCTCGAGGTGGTGTGAGGGTTTCAACTG-3′. The human placenta genomic DNA was used as the PCR template. The PCR products were digested with SacI and XhoI and inserted into the same enzyme-digested pGL3-Basic vector from Promega (Madison, WI). The pGL3-hSULT2A1 promoter mutant was generated by site-directed mutagenesis, using the forward primer 5′-GTGTTTATGCTTGATGAAAAGCTTCCTTATTGTTTTTAAGTTTGCAC-3′ and reverse primer 5′-GTGCAAACTTAAAAACAATAAGGAAGCTTTTCATCAAGCATAAACAC-3′. The identities of all cloned sequences were verified by DNA sequencing.

Human Primary Hepatocytes. Human primary hepatocytes were obtained through the Liver Tissue Procurement and Distribution System (Pittsburgh, PA).

Cell Culture and Transient Transfections. HepG2 cells were transfected using the polyethyleneimine polymer transfection agent as previously described (Ou et al., 2013), and the transfection efficiency was normalized against the

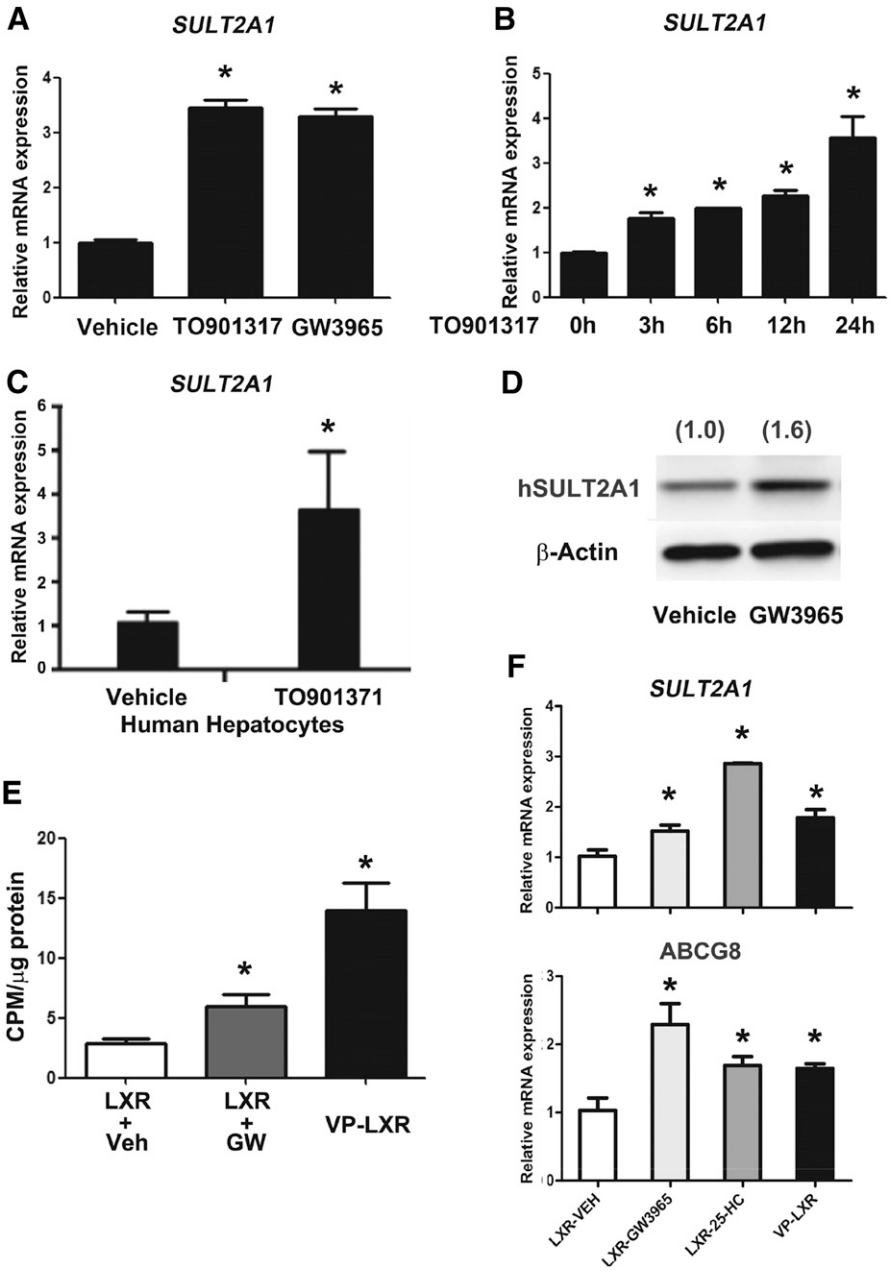

Fig. 1. LXR synthetic ligands induced the mRNA expression of SULT2A1. (A) HepG2 cells treated with synthetic LXR ligands GW3965 and TO901317 for 24 hours before cells were harvested and subjected to gene expression analysis by real-time PCR analysis. (B) HepG2 cells treated with TO901317 for indicated amounts of time were harvested, and the gene expression was analyzed by real-time PCR analysis. (C) Primary human hepatocytes were treated with TO901317 for 24 hours before gene expression analysis. (D) HepG2 cells were treated with GW3965 for 24 hours. Total protein samples were collected and subjected to Western blot analysis to detect the expression of endogenous SULT2A1 protein. The band quantifications are labeled on the top. (E) The inductions of SULT2A1 in LXRα- or VP-LXRα–transfected HepG2 cells were confirmed by enzymatic assay using hydroxysteroid dehydroepiandrosterone as the substrate. (F) The effect of the endogenous LXR ligand hydroxycholesterol (25-HC) on the expression of SULT2A1 and ATP-binding cassette G8 (ABCG8) in LXRα-transfected HepG2 cells. Treatment with GW3965 and transfection of VP-LXRα were included as positive controls. $^*P < 0.05$; $n = 3$ for each group.

β-galactosidase (β-gal) activity from the cotransfected pCMX–β-gal. For each triplicate transfection, 0.6 μg of pGL3-SULT2A1 reporter gene, 0.3 μg of pCMX-hLXRα, and 0.3 μg of pCMX–β-gal were transfected on a 48-well plate. Twenty-four hours after transfection, cells were treated with vehicle, GW3965 (10 μM), or TO901317 [*N*-(2,2,2-trifluoroethyl)-*N*-[4-[2,2,2-trifluoro-1-hydroxy-1-(trifluoromethyl)ethyl]phenyl]benzenesulfonamide] (10 μM) for 48 hours before being harvested for luciferase and β-gal assays. All transfections were performed in triplicate and repeated at least three times.

Real-Time Reverse-Transcription PCR Analysis. Total RNA was extracted with TRIzol reagent. The cDNA was synthesized from total RNA by SuperScript3 from Invitrogen (Carlsbad, CA). Aliquots of cDNA were amplified on an ABI 7300 real-time PCR system from Applied Biosystems (Foster City, CA) using the SYBR Green PCR master mix. The target mRNA expression was normalized against the cyclophilin expression.

Western Blot Analysis. Whole-cell protein extracts were prepared and measured for their protein concentrations with the protein assay kit from Pierce (Rockford, IL). One hundred micrograms of proteins were separated on 10% SDS-PAGE gels and electrotransferred onto polyvinyl difluoride transfer membranes from Invitrogen. Membranes were then incubated in Tris-buffered saline containing 0.2% (v/v) Tween 20 and 5% (w/v) fat-free dry milk at room temperature for 1 hour before incubation with the primary antibody at 4°C overnight. The primary antibody used was monoclonal anti-hSULT2A1 antibody from R&D Systems (Minneapolis, MN). The membranes were then incubated with the secondary antibody for 1 hour before signal detection by using the enhanced chemiluminescence detection system from GE Healthcare (Piscataway, NJ). The second antibody used was horseradish peroxidase–linked anti-mouse IgG. Membranes were stripped and reprobed for β-actin for the purpose of loading control.

SULT Assay. HepG2 cell cytosols were prepared by homogenizing cells in 5 mM KPO_4 buffer (pH 6.5) containing 0.25 M sucrose. A SULT assay was carried out as described previously (Kallen et al., 2002). [^{35}S]Phosphoadenosine phosphosulfate (PAPS) from PerkinElmer (Waltham, MA) was used as the sulfate donor. In brief, 20 mg/ml total cell cytosolic proteins were incubated with [^{35}S]PAPS at 37°C for 20 minutes. After the reaction, free [^{35}S]PAPS was removed by extracting with ethyl acetate. The aqueous phase was then counted in the LS6500 scintillation counter from Beckman (Palo Alto, CA) for radioactivity. Control reactions without substrate were also carried out in parallel, and each reaction was run in triplicate.

Chromatin Immunoprecipitation Assay. HepG2 cells with or without receptor transfection were treated with vehicle, TO901317, or GW3965 (10 μM each) for 24 hours before the chromatin immunoprecipitation assay as described previously (Zhou et al., 2006; Ou et al., 2011). Cell lysates were incubated overnight with 1 μg of anti-hLXRα (R&D Systems) at 4°C. Parallel samples were incubated with normal IgG as a negative control. The following PCR primers were used: SULT2A1 pro 500 F, 5′-GAGAACAGATAAAGACTGTGTGG-3′, and SULT2A1 pro 260 R, 5′-AGGGGTCATCTGAGCTTGCG-3′ for the −500 to −258 bp fragment; and SULT2A1 pro −1839 F, 5′-TTGAGACGGAGTCTCGCT-3′, and SULT2A1 pro −1640 R, 5′-ATCCTGGCTAACACGGTA-3′ for the −1839 to −1640 bp control fragment.

LXR RNA Interference. The hLXR small interfering RNAs (siRNAs) and the control scrambled siRNA were purchased from Qiagen (Valencia, CA). The sequences of siLXRα and siLXRβ are 5′-AGCAGGGCUGCAAGUGGAA-3′ and 5′-CAGAUCCGGAAGAAGAAGA-3′, respectively. The siRNA transfection was carried out using Lipofectamine 2000 (Invitrogen). The siRNAs were added to the final concentration of 20 nM during transfection. Cells were transfected for 4 hours before being replaced with fresh medium and were cultured for another 24 hours before drug treatment.

Statistical Analysis. All values are expressed as mean ± S.D. Comparisons between groups were performed using a Student's *t* test or one-way analysis of variance where appropriate. $P < 0.05$ was considered statistically significant. Linear regression was used to analyze the expression data in primary human hepatocytes.

Results

Activation of LXR Induced the Expression and Activity of SULT2A1 in Human Liver Cells. To determine whether SULT2A1 was subjected to LXR regulation in human liver cells, we treated the human hepatoma HepG2 cells with one of two synthetic LXR ligands, GW3965 or TO901317. As shown in Fig. 1A, both ligands induced the mRNA expression of SULT2A1 efficiently. We also showed that the induction of SULT2A1 mRNA by TO901317 was time-dependent (Fig. 1B). Treatment of primary human hepatocytes with TO901317 also induced the mRNA expression of SULT2A1 (Fig. 1C).

The induction of SULT2A1 at the protein level was confirmed in HepG2 cells treated with GW3965 as shown by Western blot analysis (Fig. 1D). We also showed that pharmacological or genetic activation of LXR induced SULT2A1 expression at the enzymatic level. In the pharmacological activation model, HepG2 cells were transfected with LXRα before treatment with GW3965. In the genetic activation model, HepG2 cells were transfected with the constitutively activated VP-LXRα, in which VP16 from the herpes simplex virus was fused to the N-terminal of LXRα (Uppal et al., 2007), and the cells were maintained in medium without exogenously added LXR ligands. As shown in Fig. 1E, cytosols prepared from LXRα-transfected and GW3965-treated cells or VP-LXRα–transfected cells showed elevated SULT2A1 enzymatic activity when hydroxysteroid dehydroepiandrosterone was used as the substrate. Besides the synthetic LXR ligand GW3965, we also tested the effect of the endogenous LXR ligand 25-hydroxycholesterol. As shown in Fig. 1F, treatment of LXRα-transfected HepG2 cells with 25-hydroxycholesterol induced the expression of both SULT2A1 and ATP-binding cassette G8, a known LXR target gene.

LXRα Played a Key Role in Mediating the Ligand-Responsive Induction of SULT2A1. LXR has α and β isoforms. We used LXRα and/or LXRβ siRNA knockdown in HepG2 cells to determine which LXR isoform played a more important role in regulating SULT2A1.

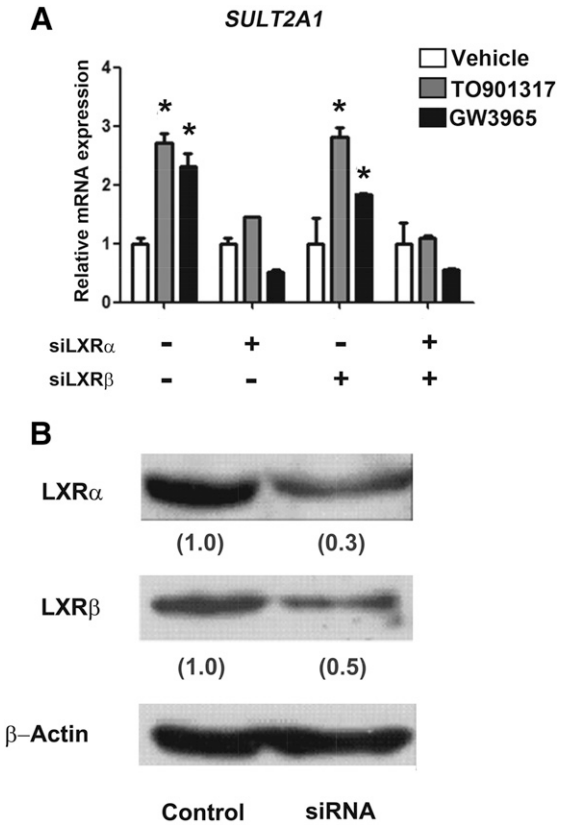

Fig. 2. LXRα played a key role in the regulation of SULT2A1. (A) HepG2 cells were transfected with control scrambled siRNA or LXRα and/or LXRβ siRNA. Twenty-four hours after the transfection, cells were treated with GW3965 or TO901317 for another 24 hours before being harvested and subjected to real-time PCR analysis to detect the expression of endogenous SULT2A1. (B) The efficiency of LXR knockdown in HepG2 cells was confirmed by Western blot analysis with the band quantifications labeled. *$P < 0.05$; $n = 3$ for each group.

As shown in Fig. 2A, knocking down of LXRα nearly abolished the inductive effects of GW3965 or TO901317 on the mRNA expression of SULT2A1, which was comparable with the effects of LXRα and LXRβ double knockdown. In contrast, knocking down of LXRβ alone had little effect on GW3965- or TO901317-responsive induction of SULT2A1. The efficiency of LXR knockdown was confirmed by Western blot analysis (Fig. 2B). These results suggested that LXRα may play a predominant role in mediating the ligand-responsive induction of SULT2A1. Because TO901317 is also known to activate PXR, another positive regulator of SULT2A1 (Sonoda et al., 2002), we cannot exclude the possibility that PXR may also play a role in regulating SULT2A1 when TO901317 is used.

The SULT2A1 Gene Promoter Was Activated by LXRα. To understand the mechanism by which LXRα regulated SULT2A1 expression, we cloned the 1250-bp 5′-flanking region of the human SULT2A1 gene and tested its regulation by LXRα using transient transfection and reporter gene assays in HepG2 cells. As shown in Fig. 3A, the 1250-bp SULT2A1 gene promoter was activated by the cotransfected LXRα upon treatment with GW3965 or TO901317.

We then used the serial deletion strategy to determine which region within the 1250-bp SULT2A1 promoter was responsible for the transactivation by LXRα. As shown in Fig. 3B, deletion from 1250 bp to 500 bp had little effect on the reporter gene activity. However, when the −500 to −258 bp region was deleted, the LXRα-responsive reporter activity was abolished, suggesting that the −500 to −258 bp region was responsible for the LXRα transactivation. Inspection of this promoter region predicted a putative direct repeat spaced by four nucleotides (DR4)-type LXR response element. Mutation of this putative DR4 in the context of the 500-bp promoter abolished the transactivation by LXRα (Fig. 3C).

We then used the chromatin immunoprecipitation assay to determine whether LXRα can be recruited to the −500 to −258 bp region of the SULT2A1 gene promoter. Indeed, in HepG2 cells, GW3965 or TO901317 treatment significantly enhanced the recruitment of LXRα to the −500 to −258 bp region of SULT2A1 gene promoter (Fig. 3C), but not the −1839 to −1640 bp negative control region (Fig. 3C). Taken together, our results suggest the SULT2A1 gene promoter is a transcriptional target of LXRα.

The Expression of SULT2A1 Was Positively Correlated with the Expression of LXRα in a Cohort of Primary Human Hepatocytes. Having established SULT2A1 as an LXRα target gene, we next hypothesized that the SULT2A1 expression level might be

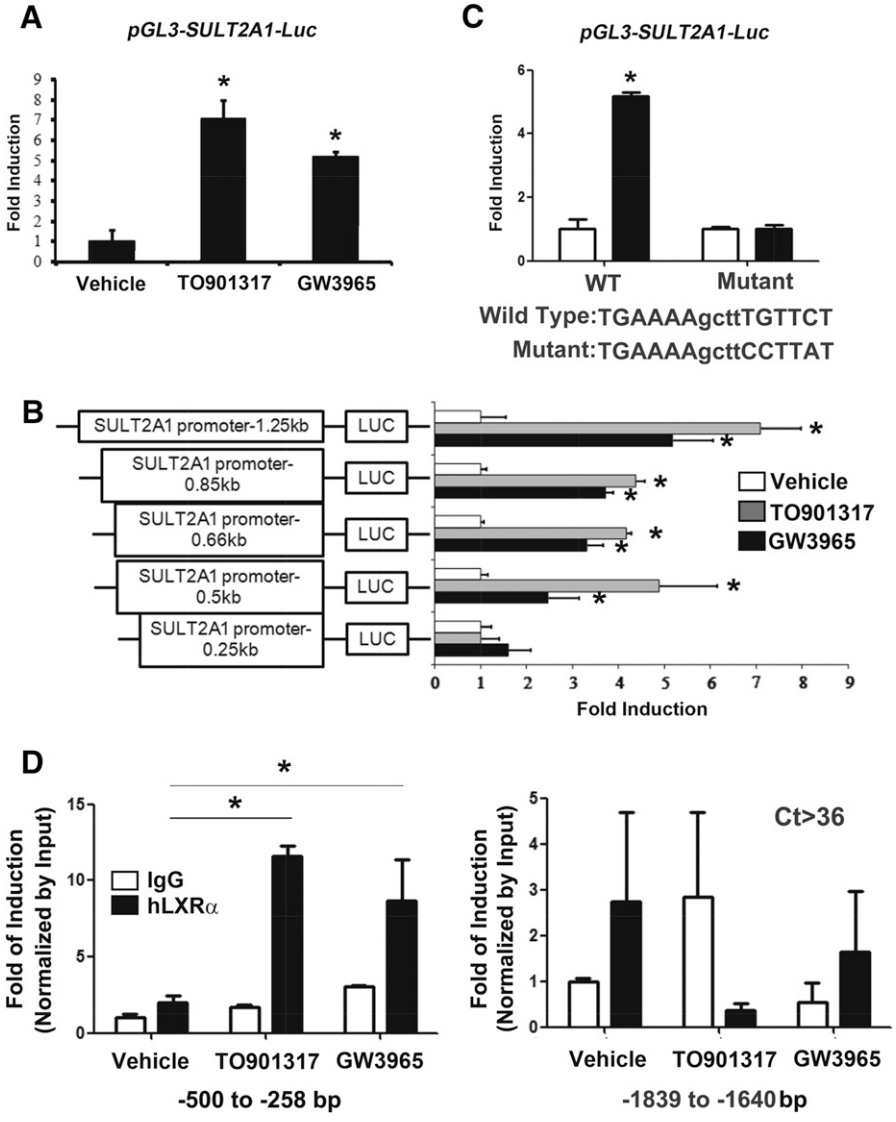

Fig. 3. LXRα transactivated the SULT2A1 gene promoter by its recruitment to the gene promoter. (A) HepG2 cells were transiently transfected with *pGL-SULT2A1* (1250-bp) reporter gene. Transfected cells were treated with vehicle or GW3965 (10 μM) or TO901317 (10 μM) for 24 hours and then harvested and assayed for luciferase and β-gal activities. The transfection efficiency was normalized against the β-gal activity. (B) The 1250-bp promoter reporter gene and its deletion mutants were evaluated for transactivation by LXRα in transient transfection and reporter gene assays. (C) HepG2 cells were transiently transfected with wild-type (WT) or mutant *pGL-SULT2A1* (500-bp) reporter gene. Transfected cells were treated with vehicle or GW3965 (10 μM) for 24 hours and then harvested and assayed for luciferase and β-gal activities. The WT and mutant DR4 sequences are labeled. (D) The recruitment of LXRα to the SULT2A1 gene promoter was confirmed by chromatin immunoprecipitation assay. Formaldehyde–cross-linked DNA was extracted from HepG2 cells and immunoprecipitated with indicated antibodies. The final DNA extracts were amplified and detected by real-time PCR using the primer pairs encompassing the −500 to −258 bp of 5′-flanking region (left panel). The −1839 to −1640 bp region was included as the negative control (right panel). *$P < 0.05$; $n = 3$ for each group. LUC, luciferase; Ct, cycle threshold.

positively correlated with the expression level of LXRα. To test this hypothesis, total RNAs from 21 independent cases of primary human hepatocytes, whose demographic information is summarized in Table 1, were collected and subjected to real-time PCR analysis for the expression of SULT2A1, LXRα, and LXRβ. Linear regression analysis showed that the expression of SULT2A1 was positively and significantly correlated with the expression of LXRα (Fig. 4A), but not LXRβ (Fig. 4B). These results were consistent with the notion that LXRα may play a predominant role in mediating the ligand-responsive induction of SULT2A1 (Fig. 2).

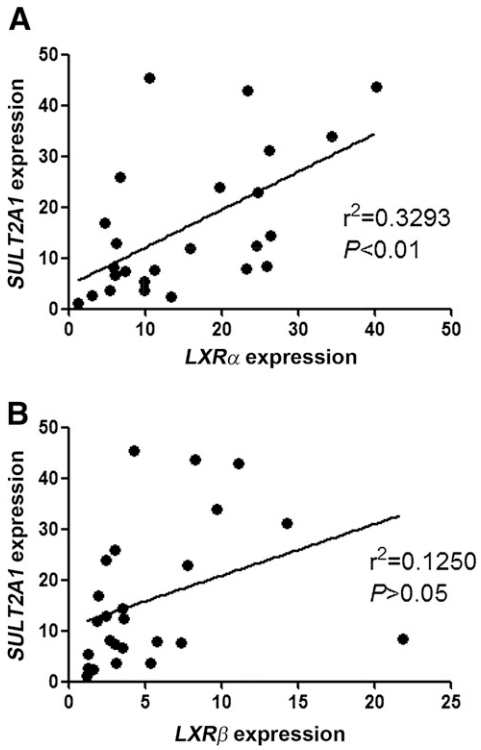

Fig. 4. The expression of SULT2A1 was positively correlated with the expression of LXRα, but not LXRβ, in a cohort of primary human hepatocytes. Twenty-one cases of primary human hepatocyte samples were analyzed for their mRNA expression of SULT2A1, LXRα, and LXRβ by real-time PCR analysis. The correlations between the expression of SULT2A1 and LXRα (A) or LXRβ (B) were analyzed by linear regression. The dots represent individual patients.

Discussion

In the current study, we showed that LXRα positively regulated SULT2A1 gene expression in human liver cells. These results were consistent with the regulation of Sult2a1 by LXR in rodents (Uppal et al., 2007). Mechanistically, LXRα regulated SULT2A1 gene expression by its recruitment to the -500 to -258 bp of the 5′-flanking region of SULT2A1 gene promoter. In a cohort of primary human hepatocytes, SULT2A1 gene expression was positively correlated with LXRα gene expression.

SULT2A1 catalyzes the sulfonation of a wide range of endogenous and exogenous compounds that have diverse effects on various physiologic functions (Repa et al., 2000; Schultz et al., 2000; Repa and Mangelsdorf, 2002; Joseph et al., 2004; Cummins et al., 2006; Zelcer and Tontonoz, 2006; Gong et al., 2007; Uppal et al., 2007, 2008). As a highly inducible phase II conjugating enzyme, the transcriptional effect of nuclear receptors on SULT2A1 gene expression and the physiologic implication of this regulation have drawn extensive interest. Within the nuclear receptor superfamily, PXR (Sonoda et al., 2002; Zhang et al., 2010), constitutive androstane receptor (Saini et al., 2004), farnesoid X receptor (Song et al., 2001), hepatocyte nuclear factor-4 (Fang et al., 2007), estrogen-related receptor-α (Seely et al., 2005), and RORs (Ou et al., 2013) have been reported as SULT2A1 transcriptional regulators in either rodents or humans. Interestingly, the regulation of SULT2A1 can be species-specific. For example, we have previously reported that the positive regulation of human SULT2A1 by RORs was opposite to the negative regulation of Sult2a1 by RORs in rodents. LXR is also known for its species-specific regulation of drug-metabolizing enzyme genes, such as its rodent-specific regulation of Sult1e1/Est (Gong et al., 2007) and Cyp7a1 (Chiang et al., 2001). Interestingly, the regulation of SULT2A1 by LXR is conserved in rodents and humans.

LXR has α and β isoforms. It is interesting to note that LXRα may play a predominant role in regulating SULT2A1, as suggested by our LXR knockdown experiments. Considering the tissue distribution of LXRα and LXRβ, it is reasonable to predict that the LXR regulation of SULT2A1 should mainly occur in LXRα-abundant tissues, such as the liver and adrenal gland. However, we cannot exclude the possibility that LXRβ may also contribute to the regulation of SULT2A1. The in vivo regulation of SULT2A1 by LXRα was evidenced by the positive correlation of SULT2A1 gene expression and LXRα gene expression in a cohort of human hepatocytes. Our results also suggested that LXRα expression had value in predicting the SULT2A1 expression level in humans. Considering the wide range of SULT2A1 substrates, it is hoped that LXRα may also have value in predicting the metabolism of SULT2A1 substrates, such as bile acids and androgens. Bile acid toxicity and increased androgen activity are the underlying mechanism for cholestasis and hormone-dependent prostate cancer, respectively. Further studies are necessary to determine whether LXRα gene expression is correlated to susceptibility to these diseases in human populations.

In summary, we report that LXRα is a transcriptional regulator of the human SULT2A1 gene. Our results also suggested that the expression of LXRα might have value in predicting the expression of SULT2A1 and susceptibility to SULT2A1-associated human diseases.

TABLE 1

Demographic information for patients from whom the primary hepatocytes were isolated

Sample No.	Age	Gender	Race	Pathology
	yr			
CTL HCS 006	34	F	Caucasian	Unknown
CTL HCS 007	21	M	Caucasian	Unknown
CTL HCS 012	26	F	Caucasian	Unknown
CTL HCS 016	46	F	African-American	Unknown
CTL HCS 022	55	M	African-American	Unknown
CTL HCS 009	22	F	Caucasian	Unknown
CTL HL-263	44	F	Caucasian	Unknown
HPH 014 CTL	55	F	Caucasian	Unknown
HPH 012 CTL	33	M	Caucasian	Unknown
1132	43	F	Caucasian	Donor
1425	47	M	Caucasian	Metastatic colon cancer
1446	37	M	Caucasian	Pancreatic cancer
1447	36	F	Caucasian	FNH
1448	45	F	Caucasian	Metastatic colon cancer
1466	68	F	Caucasian	Colon cancer
1467	52	F	Caucasian	Metastatic colon cancer
1468	38	M	Caucasian	Metastatic colon cancer
1469	46	F	Caucasian	Cardiac arrest
1478	60	F	Caucasian	Metastatic colon cancer
1482	24	F	Caucasian	FNH
1486	57	M	Caucasian	Metastatic colon cancer

F, female; FNH, focal nodular hyperplasia; M, male.

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Authorship Contributions

Participated in research design: Ou, Jiang, Hu, Y. Huang, Xu, Ren.

Conducted experiments: Ou, Jiang, Hu, Y. Huang, Xu, Ren.

Contributed new reagents or analytic tools: Ou, Jiang, Hu, Y. Huang, Xu, Ren.

Performed data analysis: Ou, Li, Liu, Xie, M. Huang.

Wrote or contributed to the writing of the manuscript: Ou, Li, Liu, Xie, M. Huang.

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