Transcriptional Regulation of Human Hydroxysteroid Sulfotransferase SULT2A1 by LXRα

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Running title: LXR regulates SULT2A1

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Abbreviations:

ChIP, chromatin immunoprecipitation; LXR, liver X receptor; LXRE, LXR response element; SULT2A1, hydroxysteroid sulfotransferase
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 (DHEA) 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 (ChIP) assays showed that LXRα transactivated the SULT2A1 gene promoter through its specific binding to the -500 to -258 bp region of the SULT2A1 gene promoter. LXR siRNA knock-down 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 the 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 expresses ubiquitously (Tontonoz et al., 2003). When activated by its endogenous (Janowski et al., 1996; Forman et al., 1997; Lehmann et al., 1997; Song et al., 2000) or synthetic ligands (Schultz et al., 2000, Collins et al. 2002), LXR forms heterodimers with the retinoid X receptor (RXR), and then binds to LXR response elements (LXREs) in the promoters of LXR target genes.

In humans, LXR regulates a wide range of genes, including ATP-binding cassette (ABC) transporter isoforms A1, G1, G5 and G8), apolipoprotein E (ApoE), cholestereoler transfer protein (CETP), lipoprotein lipase (LPS), fatty acid synthase (FAS), and sterol regulatory element binding protein-1c (SREBP-1c) (Edwards et al., 2002). Through its gene regulatory activities, LXR is involved in diverse physiological functions in rodents, including cholesterol and lipid metabolism (Repa et al., 2000; 2002; Schultz et al., 2000), anti-inflammation (Zelcer et al., 2006; Joseph et al., 2004), 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 highly expressed in the liver and adrenal gland (Falany et al., 1997), catalyzes the metabolism of bile acids (Radomsinska et al., 1990), hydroxysteroid dehydroepiandrosterone (DHEA) (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 (CAR) (Saini et al., 2004), hepatocyte nuclear factor-4 (Fang et al., 2007), farnesoid X receptor (Song et al., 2001), estrogen receptor-related receptor-a (Seely et al., 2005), and RORα 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 anti-cholestatic activity of LXR (Uppal et al., 2007). However, the regulatory effect of LXR on the human SULT2A1 gene expression remains unclear.

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 was synthesized in-house as previously described (Collins et al., 2002). All other chemicals, if not specified, were purchased from Sigma (St. Louis, MO).

Plasmid constructs

The pGL3-SULT2A1 promoter reporters that contain 1250-bp of the SULT2A1 gene promoter or its deletion mutants were cloned by 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’-GTGCAAACTTTAAAAACAATAAGGAAGCTTTTCATCAAGCATAAACAC-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 \( \beta \)-galactosidase (\( \beta \)-gal) activity from the co-transfected pCMX-\( \beta \)-gal. For each triplicate transfection, 0.6 \( \mu \)g of pGL3-SULT2A1 reporter gene, 0.3 \( \mu \)g of pCMX-hLXR\( \alpha \), and 0.3 \( \mu \)g of pCMX-\( \beta \)-gal were transfected on a 48-well plate. Twenty-four hours after transfection, cells were treated with vehicle, GW3965 (10 \( \mu \)M) or TO901317 (10 \( \mu \)M) for 48 h before being harvested for luciferase and \( \beta \)-gal assays. All transfections were performed in triplicate and repeated at least three times.

Real-time RT-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% (vol/vol) Tween 20 and 5% (wt/vol) fat-free dry milk at room temperature for 1 h before incubation with the primary antibody at 4 C overnight. The primary antibody used was monoclonal anti-hSULT2A1 antibody (Catalog No. Mab5828) from R&D Systems (Minneapolis, MN). The membranes were then incubated with the secondary antibody for 1 h before signal detection by using the enhanced chemiluminescence detection system from GE Health Healthcare (Piscataway, NJ). The second antibody used was horseradish peroxidase-linked anti-mouse IgG. Membranes were stripped and re-probed for β-actin for the purpose of loading control.

Sulfotransferase assay

HepG2 cell cytosols were prepared by homogenizing cells in 5 mM KPO4 buffer (pH 6.5) containing 0.25 M sucrose. A sulfotransferase assay was carried out as described previously (Kallen et al. 2002). [35S]-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 [35S]-PAPS
at 37 C for 20 min. After the reaction, free \[^{35}\text{S}]\text{-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 (ChIP) assay**

HepG2 cells with or without receptor transfection were treated with vehicle, TO901317 or GW3965 (10 \(\mu\text{M}\) each) for 24 h before the ChIP assay as described previously (Ou et al., 2011; Zhou et al., 2006). Cell lysates were incubated overnight with 1 \(\mu\text{g}\) of anti-hLXR\(\alpha\) (catalog # PP-PPZ0412-00) from 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’-

GAGAACAGATAAAGACTGTGG-3’, and SULT2A1 pro 260 R, 5’-

AGGGGTCATCTGAGCTTGCG-3’ for the 500-260 bp fragment, and SULT2A1 pro -1839 F, 5’- TTGAGACGGGAGTCTCGCT-3’, and SULT2A1 pro -1640 R, 5’-

ATCCTGGCTAACCACGGTA-3’ for the 1839-1640 bp control fragment.

**LXR RNA interference (siRNA)**

The hLXR siRNAs and the control scrambled siRNA were purchased from QIAGEN. The sequences of siLXR\(\alpha\) and siLXR\(\beta\) are 5’-AGCAGGGCUGCAAGUGAA-3 and 5’-CAGAUCCGGAAGAAGAAGA-3, respectively. The control scrambled siRNA (catalog no. 1027280) was purchased from QIAGEN. 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 h before being replaced with fresh medium and were cultured for another 24 h before drug treatment.

**Statistical analysis**

All values were expressed as mean ± SD. Comparisons between groups were performed using a Student t-test or one-way ANOVA 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 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 treating with GW3965. In the genetic activation model, HepG2 cells were transfected with the constitutively activate 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 cell or VP-LXRα-transfected cells showed elevated SULT2A1 enzymatic
activity when using DHEA as the substrate. Besides the synthetic LXR ligand GW3965, we also tested the effect of the endogenous LXR ligand 25-hydroxycholesterol (25-HC). As shown in Fig. 1F, treatment of LXRα-transfected HepG2 cells with 25-HC induced the expression of both SULT2A1 and ABCG8, 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. 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 knock-down. 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. Since 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 was used.

**The SULT2A1 gene promoter was activated by LXRα**
To understand the mechanism by which LXR\(\alpha\) regulated SULT2A1 expression, we cloned the 1250-bp 5' franking region of the human SULT2A1 gene and tested its regulation by LXR\(\alpha\) using transient transfection and reporter gene assay in HepG2 cells. As shown in Fig. 3A, the 1250-bp SULT2A1 gene promoter was activated by the co-transfected LXR\(\alpha\) upon the treatment of 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 LXR\(\alpha\). As shown in Fig. 3B, deletion from 1250 bp to 500 bp had little effect on the reporter gene activity. However, when the 500-258 bp region was deleted, the LXR\(\alpha\)-responsive reporter activity was abolished, suggesting the 500-258 bp region was responsible for the LXR\(\alpha\) 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 LXR\(\alpha\) (Fig. 3C).

We then used ChIP assay to determine whether LXR\(\alpha\) can be recruited to the 500-258 bp region of the SULT2A1 gene promoter. Indeed, in HepG2 cells, GW3965 or TO901317 treatment significantly enhanced the recruitment of LXR\(\alpha\) to the 500-258 bp region of SULT2A1 gene promoter (Fig. 3C), but not the 1839-1640 bp negative control region (Fig. 3C). Taken together, our results suggested the SUL2A1 gene promoter is a transcriptional target of LXR\(\alpha\).
The expression of SULT2A1 was positively correlated with the expression of LXRα in a cohort of primary human hepatocytes

Having established SULT2A1 as a LXRα target gene, we next hypothesized that the SULT2A1 expression level might be 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).
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 500-258 bp of 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 physiological functions (Repa et al., 2000; Repa et al., 2002; Schultz et al., 2000; Zelcer et al., 2006; Joseph et al., 2004; Uppal et al., 2007; Uppal et al., 2008; Cummins et al., 2006; Gong et al., 2007). As a highly inducible phase II conjugating enzyme, the transcriptional effect of nuclear receptors on SULT2A1 gene expression and the physiological implication of this regulation have drawn extensive interest. Within the nuclear receptor superfamily, PXR (Sonoda et al., 2002; Zhang et al., 2010), CAR (Saini et al., 2004), FXR (Song et al., 2001), HNF4 (Fang et al., 2007), ERRα (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 two isoforms. It is interesting to note that LXRα may play a predominant role in regulating SULT2A1 as suggested by our LXR knock-down 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 \textit{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 a value in predicting the SULT2A1 expression level in humans. Considering the wide range of SULT2A1 substrates, it is hoped that LXRα may also have a 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 the susceptibility to these diseases in human populations.

In summary, we report that LXRα is a transcriptional regulator of the human
DMD #58479

SULT2A1 gene. Our results also suggested that the expression of LXRα might have a value in predicting the expression of SULT2A1 and susceptibility to SULT2A1 associated human diseases.
DMD #58479

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Authorship Contributions:

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

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

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

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

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


expression is important for macrophage survival and the innate immune response. Cell 119:299–309.


Footnotes:
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Figure legends

Figure 1. LXR synthetic ligands induced the mRNA expression of SULT2A1. (A) HepG2 cells treated with synthetic LXR ligands GW3965 and TO901317 for 24 h 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 h before gene expression analysis. (D) HepG2 cells were treated with GW3965 for 24 h. 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 DHEA as the substrate. (F) The effect of endogenous LXR ligand 25-hydroxycholesterol (25-HC) on the expression of SULT2A1 and 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.

Figure 2. LXRα played a key role in the regulation of SULT2A1. (A) HepG2 cells were transfected with control scrambled siRNA (siControl) or LXRα and/or LXRβ siRNA. Twenty four hours after the transfection, cells were treated with GW3965 or TO901317 for another 24 h 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.

Figure 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 h, 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 its 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 h, 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 (ChIP) 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.
Figure 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 hepatocytes 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.
Table 1. Demographic information of patients from which the primary hepatocytes were isolated

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Figure 1

A

**SULT2A1**

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<td><strong>6h</strong></td>
<td><img src="image7" alt="Bar graph A 6h" /></td>
<td><img src="image8" alt="Bar graph A TO901317 6h" /></td>
<td><img src="image9" alt="Bar graph A GW3965 6h" /></td>
</tr>
<tr>
<td><strong>12h</strong></td>
<td><img src="image10" alt="Bar graph A 12h" /></td>
<td><img src="image11" alt="Bar graph A TO901317 12h" /></td>
<td><img src="image12" alt="Bar graph A GW3965 12h" /></td>
</tr>
<tr>
<td><strong>24h</strong></td>
<td><img src="image13" alt="Bar graph A 24h" /></td>
<td><img src="image14" alt="Bar graph A TO901317 24h" /></td>
<td><img src="image15" alt="Bar graph A GW3965 24h" /></td>
</tr>
</tbody>
</table>

B

**SULT2A1**

<table>
<thead>
<tr>
<th>Relative mRNA expression</th>
<th>TO901317</th>
<th>0h</th>
<th>3h</th>
<th>6h</th>
<th>12h</th>
<th>24h</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vehicle</strong></td>
<td><img src="image16" alt="Bar graph B Vehicle" /></td>
<td><img src="image17" alt="Bar graph B 0h" /></td>
<td><img src="image18" alt="Bar graph B 3h" /></td>
<td><img src="image19" alt="Bar graph B 6h" /></td>
<td><img src="image20" alt="Bar graph B 12h" /></td>
<td><img src="image21" alt="Bar graph B 24h" /></td>
</tr>
<tr>
<td><strong>TO901317</strong></td>
<td><img src="image22" alt="Bar graph B TO901317" /></td>
<td><img src="image23" alt="Bar graph B 0h" /></td>
<td><img src="image24" alt="Bar graph B 3h" /></td>
<td><img src="image25" alt="Bar graph B 6h" /></td>
<td><img src="image26" alt="Bar graph B 12h" /></td>
<td><img src="image27" alt="Bar graph B 24h" /></td>
</tr>
</tbody>
</table>

C

**SULT2A1**

<table>
<thead>
<tr>
<th>Relative mRNA expression</th>
<th>Vehicle</th>
<th>TO901317</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Human Hepatocytes</strong></td>
<td><img src="image28" alt="Bar graph C Vehicle" /></td>
<td><img src="image29" alt="Bar graph C TO901317" /></td>
</tr>
</tbody>
</table>

D

**hSULT2A1**

<table>
<thead>
<tr>
<th>Relative mRNA expression</th>
<th>Vehicle</th>
<th>GW3965</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>(1.0)</strong></td>
<td><img src="image30" alt="Bar graph D Vehicle" /></td>
<td><img src="image31" alt="Bar graph D GW3965" /></td>
</tr>
<tr>
<td><strong>(1.6)</strong></td>
<td><img src="image32" alt="Bar graph D Vehicle" /></td>
<td><img src="image33" alt="Bar graph D GW3965" /></td>
</tr>
</tbody>
</table>

**β-Actin**

<table>
<thead>
<tr>
<th>Relative mRNA expression</th>
<th>Vehicle</th>
<th>GW3965</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>β-Actin</strong></td>
<td><img src="image34" alt="Bar graph D Vehicle" /></td>
<td><img src="image35" alt="Bar graph D GW3965" /></td>
</tr>
</tbody>
</table>

E

**CYP3A**

<table>
<thead>
<tr>
<th>Relative mRNA expression</th>
<th>LXR</th>
<th>LXR</th>
<th>VFP-LXR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vehicle</strong></td>
<td><img src="image36" alt="Bar graph E Vehicle" /></td>
<td><img src="image37" alt="Bar graph E LXR" /></td>
<td><img src="image38" alt="Bar graph E VFP-LXR" /></td>
</tr>
<tr>
<td><strong>LXR</strong></td>
<td><img src="image39" alt="Bar graph E LXR" /></td>
<td><img src="image40" alt="Bar graph E LXR" /></td>
<td><img src="image41" alt="Bar graph E VFP-LXR" /></td>
</tr>
<tr>
<td><strong>LXR</strong></td>
<td><img src="image42" alt="Bar graph E LXR" /></td>
<td><img src="image43" alt="Bar graph E LXR" /></td>
<td><img src="image44" alt="Bar graph E VFP-LXR" /></td>
</tr>
<tr>
<td><strong>VFP-LXR</strong></td>
<td><img src="image45" alt="Bar graph E VFP-LXR" /></td>
<td><img src="image46" alt="Bar graph E VFP-LXR" /></td>
<td><img src="image47" alt="Bar graph E VFP-LXR" /></td>
</tr>
</tbody>
</table>

F

**SULT2A1**

<table>
<thead>
<tr>
<th>Relative mRNA expression</th>
<th>LXR</th>
<th>LXR</th>
<th>VFP-LXR</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vehicle</strong></td>
<td><img src="image48" alt="Bar graph F Vehicle" /></td>
<td><img src="image49" alt="Bar graph F LXR" /></td>
<td><img src="image50" alt="Bar graph F VFP-LXR" /></td>
</tr>
<tr>
<td><strong>LXR</strong></td>
<td><img src="image51" alt="Bar graph F LXR" /></td>
<td><img src="image52" alt="Bar graph F LXR" /></td>
<td><img src="image53" alt="Bar graph F VFP-LXR" /></td>
</tr>
<tr>
<td><strong>LXR</strong></td>
<td><img src="image54" alt="Bar graph F LXR" /></td>
<td><img src="image55" alt="Bar graph F LXR" /></td>
<td><img src="image56" alt="Bar graph F VFP-LXR" /></td>
</tr>
<tr>
<td><strong>VFP-LXR</strong></td>
<td><img src="image57" alt="Bar graph F VFP-LXR" /></td>
<td><img src="image58" alt="Bar graph F VFP-LXR" /></td>
<td><img src="image59" alt="Bar graph F VFP-LXR" /></td>
</tr>
</tbody>
</table>
Figure 2

A

SULT2A1

Vehicle
TO901317
GW3966

siLXRα - + - +

siLXRβ - - + +

Relative mRNA expression

B

LXRα (1.0) (0.3)

LXRβ (1.0) (0.5)

β-Actin

Control siRNA
Figure 3

A

B

Wild Type: TGAAAGGcttTGGTCT
Mutant: TGAAAGGcttCTTAT

D

C

Fold Induction

Vehicle TO901317 GW3965

Fold Induction

Vehicle TO901317 GW3965

-500 to -258 bp

-1839 to -1640 bp

Vehicle TO901317 GW3965

-15

-10

-5

0

-15

-10

-5

0

IgG hLXRα

Normalized by Input

Normalized by Input

Ct>36
Figure 4

A

SULT2A1 expression

$\text{LXR}_\alpha$ expression

$r^2 = 0.3293$

$P < 0.01$

B

SULT2A1 expression

$\text{LXR}_\beta$ expression

$r^2 = 0.1250$

$P > 0.05$