Liver Receptor Homolog-1 Regulates Organic Anion Transporter 2 and Docetaxel Pharmacokinetics[§]

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ABSTRACT

Organic anion transporter 2 (OAT2/SLC22A7) is an uptake transporter that plays an important role in drug disposition. Here, we investigate a potential role of liver receptor homolog-1 (Lrh-1) in regulation of Oat2 and docetaxel pharmacokinetics. Hepatoma cells (Hepa1-6 and HepG2 cells) were transfected with Lrh-1/LRH-1 expression vector or siRNA. The relative mRNA and protein levels of Oat2/OAT2 in the cells or livers of Lrh-1^{hep-/-} mice were determined by qPCR and Western blotting, respectively. Transcriptional regulation of Oat2/OAT2 by Lrh-1/LRH-1 was investigated using luciferase reporter, mobility shift, and chromatin immunoprecipitation (ChIP) assays. Pharmacokinetic studies were performed with wild-type (Lrh-1^{fl/fl}) and Lrh-1^{hep-/-} mice after intraperitoneal injection of docetaxel. Overexpression of Lrh-1 in Hepa1-6 cells led to significant increases in Oat2 mRNA and

protein. Consistently, Lrh-1 knockdown caused decreases in Oat2 mRNA and protein, as well as reduced cellular uptake of PGE₂, a prototypical substrate of Oat2. Similarly, an activation effect of LRH-1 on OAT2 expression was observed in HepG2 cells. In addition, the levels of Oat2 mRNA and protein were markedly reduced in Lrh-1^{hep-/-} mice. Lrh-1/LRH-1 induced the transcription of Oat2/OAT2 in luciferase reporter assays. Truncation analysis revealed a potential Lrh-1 response element (–716- to –702-bp) in Oat2 promoter. Direct binding of Lrh-1 to this response element was confirmed by mobility shift and ChIP assays. Furthermore, systemic exposure of docetaxel was upregulated in Lrh-1^{hep-/-} mice due to reduced hepatic uptake. In conclusion, Lrh-1 transcriptionally regulates Oat2, thereby impacting tissue uptake and pharmacokinetics of Oat2 substrates.

Introduction

Docetaxel is a chemotherapeutic agent approved for the treatment of various types of cancers, including breast cancer, non-small cell lung cancer, squamous cell carcinoma of the head and neck, gastric adenocarcinoma, and androgen-independent metastatic prostate cancer (Connolly et al., 2011). The pharmacokinetic profile of docetaxel is subjected to a large interindividual variability, with up to 10-fold differences in drug clearance even in patients with normal hepatic function (Baker et al., 2006). The causes of this variability are probably multifactorial, including the factors affecting transport and metabolism of docetaxel. The membrane transporter systems have been shown to modulate the pharmacokinetics of docetaxel (Shirakawa et al., 1999; Hopper-Borge et al., 2004; Ehrlichova et al., 2005; de Graan et al., 2012). In particular, hepatic uptake of docetaxel is mediated by several members of solute carrier (SLC) family transporters including the organic anion transporter 2 (OAT2) (Franke et al., 2010; de Graan et al., 2012).

OAT2 is an uptake transporter also known as the member 7 of the solute carrier 22A family (SLC22A7). OAT2 is highly expressed in the liver and kidney, thus playing an important role in determining pharmacokinetics and drug efficacy (Sekine et al., 1998; Kobayashi et al., 2005a). The substrates of OAT2 include anti-HIV drugs (e.g., zidovudine), anticancer drugs (e.g., docetaxel and methotrexate), antibiotics (e.g., erythromycin), and anti-inflammatories (e.g., salicylate) (Sun et al., 2001; Kobayashi et al., 2005b; Baker et al., 2006; Burger et al., 2011; Cheng et al., 2012). OAT2 expression is reported to be a critical determinant to individual response to 5-fluorouracil-based chemotherapy (Nishino et al., 2013). It is also involved in transport of many endogenous compounds such as uric acid, creatinine, and prostaglandin E₂ (PGE₂) (Anzai et al., 2006; Sato et al., 2010; Shen et al., 2015). Genetic deficiency of OAT2 has been associated with hyperuricemia disorders (Anzai and Endou, 2007).

Liver receptor homolog-1 (LRH-1/NR5A2) is a nuclear receptor significantly expressed in various tissues including the drug-eliminating organs liver, kidney, and intestine (Fayard et al., 2004). LRH-1 receptor plays a critical role in regulation of metabolic enzymes and transporters (Kobayashi et al., 2005). For instance, LRH-1 promotes the expression of CYP7A1/CYP8B1 (two bile acid-synthesizing enzymes) and CYP19A1 (a critical enzyme for conversion of androgen to estrogen) (del Castillo-Olivares and Gil, 2000; Lu et al., 2000; Chand et al., 2011). It activates the transcription of many ABC transporters such as the bile

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ABBREVIATIONS: Alb, albumin; ChIP, chromatin immunoprecipitation assay; EMSA, electrophoretic mobility shift assay; HNF, hepatocyte nuclear factor; Lrh-1/LRH-1, mouse/human liver receptor homolog-1; Oat2/OAT2, mouse/human organic anion transporter 2; PGE₂, prostaglandin E₂; siLrh-1, short interfering RNA for Lrh-1; SLC, solute carrier; siRNA, short interfering RNA; UPLC-QTOF/MS, ultraperformance liquid chromatography/quadrupole time-of-flight mass spectrometry.

salt export pump, multidrug resistance protein 3, and ABCG5/G8 (Freeman et al., 2004). Therefore, LRH-1 is regarded as a key regulator of drug-detoxifying genes.

Due to important roles of OAT2 in biology and pharmacology, the regulatory mechanisms for its expression are of particular interest. Previous studies have shown that hepatocyte nuclear factor-1 alpha (HNF-1 α) and HNF-4 α stimulate the expression of OAT2 by binding to a DR-1 site in the promoter (Popowski et al., 2005; Maher et al., 2006), whereas several receptors (including farnesoid X receptor, constitutive androstane receptor, and pregnane X receptor) downregulate OAT2 via a direct or indirect mechanism (Jigorel et al., 2006; Shen et al., 2015). However, it remains unclear whether and how LRH-1 regulates OAT2 expression.

In this study, we unravel a critical role for Lrh-1 receptor in regulation of Oat2 and docetaxel pharmacokinetics. We first showed that Lrh-1/LRH-1 induced the mRNA and protein expression of Oat2/OAT2 in hepatoma cells. Accordingly, downregulation of Oat2 by Lrh-1 silencing led to significant decreases in cellular uptake of PGE2 and docetaxel, two known substrates of Oat2. Through a combination of promoter analysis, mobility shift, and chromatin immunoprecipitation (ChIP) assays, we demonstrated that Lrh-1 transactivated Oat2 by its specific binding to -716 to -702-bp region within the gene promoter. Furthermore, conditional deletion of hepatic Lrh-1 in mice (Lrh-1^{hep-/-} mice) led to marked downregulation of Oat2 and altered docetaxel pharmacokinetics.

Materials and Methods

Materials. Rabbit polyclonal LRH-1 (cat. no. AP21181C, 1:1000 dilution) antibody for Western blot analysis was purchased from Abgent (San Diego, CA). Rabbit polyclonal OAT2 (cat. no. ab186476, 1:1000 dilution) antibody, rabbit monoclonal GAPDH (cat. no. ab181602, 1:5000 dilution) antibody, and mouse monoclonal LRH-1 (cat. no. ab41901) antibody for ChIP assay were obtained from Abcam (Cambridge, MA). Prostaglandin E₂ (PGE₂) was purchased from Tocris (Bristol, UK). Docetaxel was obtained from Shanghai TAUTO Biotech (Shanghai, China). The transfection reagent jetPRIME was purchased from Polyplus Transfection (Illkirch, France). Dulbecco's modified Eagle's medium (high glucose) was purchased from Sigma-Aldrich (St. Louis, MO). Fetal bovine serum and trypsin were obtained from Hyclone (Logan, UT).

Cell Lines and Cell Culture. Hepa1-6 cells (cat. no. CRL-1830; ATCC, Manassas, VA), HepG2 cells (cat. no. HB-8065; ATCC, Rockville, MD) and HEK-293T cells (cat. no. CRL-11268; ATCC) were grown at 37°C in a humidified atmosphere (5% CO₂) in plastic culture flasks. The growth medium was Dulbecco's modified Eagle's medium (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (Hyclone). The medium was changed every other day, and the culture was split every 5 days.

Animal Studies. Lrh-1 floxed mice (a kind gift from Dr. Bruce Murphy, University of Montreal, Canada) were crossed with serum albumin (Alb)-Cre mice (Model Animal Research Center of Nanjing University, China) and then further intercrossed to generate liver-specific Lrh-1 knockout (named Lrh-1^{hep-/-} mice) and wild-type (Lrh-1^{fl/fl}) mice on a pure C57BL/6J background. All mice, receiving food and water ad libitum, were housed in a temperature-controlled room with a 12-hour light/12-hour dark cycle. All mice were fasted overnight prior to each study. For the first set of study, wild-type and Lrh-1^{hep-/-} mice (8–10 weeks of age, male, $n = 5$ per group) were euthanized, and the livers were isolated, snap-frozen, and stored at -80°C until processed for mRNA and protein analyses.

For the second set of pharmacokinetic study, two groups of male mice (wild-type and Lrh-1^{hep-/-}) were intraperitoneally injected with a single dose of docetaxel (10 mg/kg). Six mice were euthanized at each time point to collect blood and livers. Blood was centrifuged at 5000 g for 10 minutes to obtain the plasma sample. The plasma and liver samples were processed for drug quantification as described in our previous publication (Zhang et al., 2018). Drug quantification was performed using the UPLC-QTOF/MS system (Waters, Milford, MA). Pharmacokinetic data were analyzed using the Bailer's approach as previously described (Zhou et al., 2008) with Microsoft Excel Visual Basic. All experimental

procedures were approved by Jinan University Institutional Animal Care and Use committee and were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Plasmid Construction and Cell Transfection. Mouse Lrh-1 (GenBank accession number: NM_030676.3) and human LRH-1 (GenBank accession number: NM_205860.2) gene (full length) were synthesized and cloned into the EcoRI and HindIII sites of the expression vector pcDNA3.1(-) (Invitrogen). Mouse Oat2 proximal promoter (~2 kb; -2000/+89; +1 indicates the transcription initiation site) and human OAT2 proximal promoter (-2000/+28) were synthesized and cloned into the NheI and HindIII sites of the blank pGL4.11 vector (Promega, Madison, WI), respectively. Shorter promoter sequences (-1.1, -0.4, and -0.15 kb) were prepared by PCR reactions from the -2000/+89 Oat2 promoter plasmid using primers containing a NheI or a HindIII restriction enzyme site (Table 1). The obtained fragments were cloned into the pGL4.11 plasmid. The siRNAs for Lrh-1 (siLrh-1) and negative control were obtained from Transheep (Transheep, Shanghai, China) (siRNA sequences are provided in Table 1). All constructs were verified by DNA sequencing. After being transformed into *Escherichia coli* JM109 cells, plasmids were isolated and purified using the EasyPure HiPure Plasmid MiniPrep kit (TransGen Biotech, Beijing, China) according to the manufacturer's instructions. Cell transfection was performed using the jetPRIME transfection reagent according to the instruction manual.

qPCR Assay. Total RNA from mouse liver samples or cells was isolated using the TRIzol method. cDNAs were synthesized from total RNA with SuperScript II reverse transcriptase (Invitrogen) and random hexamer primers (Roche, Basel, Switzerland). qPCR reactions were performed following our published procedures (Zhang et al., 2018). All primer sequences are summarized in Table 1.

Luciferase Reporter Assay. Luciferase reporter assay was performed following our published procedures with minor modifications (Lu et al., 2017). In brief, HEK-293T cells were seeded in 48-well plates (Corning Life Sciences, Acton, MA) at a density of 5×10^4 cells/well. The next day, cells were transfected with 250 ng of Oat2 luciferase (firefly) reporter plasmid, 25 ng of pRL-TK vector (an internal control with renilla luciferase gene), and a fixed amount (125, 250, or 500 ng) of Lrh-1 expression vector. A control experiment was performed in the absence of Lrh-1 expression vector. For Lrh-1-silencing experiments, siRNA plasmid (50 nM) was cotransfected with the reporter plasmid. Cell transfection was performed using the jetPRIME transfection reagent (Polyplus Transfection). After 24-hour transfection, cells were harvested in the passive lysis buffer. The cell lysate was assayed for luciferase activities using the Dual-Luciferase Reporter Assay System and GloMax 20/20 luminometer (Promega). Data were analyzed exactly as previously described (Lu et al., 2017).

Western Blotting. Western blotting were performed as previously described (Lu et al., 2017). In brief, mouse liver or whole cell (40 μ g) lysates were separated on a 10% sodium dodecyl sulfate-polyacrylamide gel followed by transfer to a polyvinylidene difluoride membrane. The membrane was incubated with the primary antibody overnight at 4°C and then with the secondary antibody for 1 hour at room temperature. Proteins were visualized with enhanced chemiluminescence and analyzed by the Quantity One software. Relative protein levels were normalized to Gapdh.

Electrophoretic Mobility Shift Assay and ChIP Assay. EMSA and ChIP assays were performed following our published procedures with minor modifications (Lu et al., 2017). For EMSA, the nuclear extracts of Hepa1-6 cells were incubated with the probes (Table 1). For ChIP, mouse liver was fixed in 1% formaldehyde and digested with micrococcal nuclease. The sheared chromatin was immunoprecipitated overnight with anti-Lrh-1 (Abcam) or normal mouse IgG (control) at 4°C. The purified DNAs were analyzed by qPCR with indicated primers (Table 1).

Cellular Uptake Study. Hepa1-6 cells were seeded into six-well plates and cultured in Dulbecco's modified Eagle's medium (10% fetal bovine serum). Twelve hours later, cells were transfected with siLrh-1-3 plasmid (50 nM) or an equal amount of siNC for negative control. Thirty-six hours after transfection, the cells were used for cellular uptake experiments. Cells were incubated with PGE₂ (a known endogenous substrate for Oat2) or docetaxel (a known drug substrate for Oat2, at a series of concentrations) dissolved in Hanks' balanced salt solution. At each time point, the incubation solution was aspirated. After being washed with ice-cold PBS twice, the cells were disrupted and solubilized with 0.4 ml 50% methanol. After centrifugation (4°C) at 13,000 g for 15 minutes, the supernatant was collected and subjected to UPLC-QTOF/MS analysis.

TABLE 1
Oligonucleotides used in this study

	Forward (5' to 3')	Reverse (5' to 3')
qRT-PCR		
hLRH-1	CTTTGTCCCGTGTGTGGAGAT	GTCGGCCCTTACAGCTTCTA
hOAT2	AGCCTCCGTCAGCTATGTAAT	CATCGCCAGTCCCGTATCA
hGAPDH	CATGAGAAGTATGACAACAGCCT	AGTCCTTCCACGATACCAAAGT
mLrh-1	GAACGTGTCCAAACCAAAAAAGG	CTTCCAGCTTCATCCCAAC
mOat2	CAACTGCGGAATCTGGTGCT	ATCAGGCAGGGCACAATGATG
mOatp1b2	CCTGAGAAGTGTCCGCATAAC	GTCCGAGTGGCAGTAAGAAAG
mOatp1a1	GTGCATACCTAGCCAAATCACT	CCAGGCCATAACACACATC
mOatp1a4	GCTTTTCCAAGATCAAGGCATT	CGTGGGGATACCGAATTGTCT
mCyp8b1	GCCTTCAAGTATGATCGGTTCT	GATCTTCTGCCCCGACTGTAGA
mPpib	TCCACACCCTTTTCCGGTCC	CAAAAGGAAGACGACGGAGC
Reporter plasmid		
hOAT2(-1100/+28)	CGCTTTTCTCAGGGATCCCAGCCTGGAG	CCTCAAAGCCCCATGCTGCTCACCCA
hOAT2(-300/+28)	GGCCCCAGGCCCCAGGGCCTCTCTT	CCTCAAAGCCCCATGCTGCTCACCCA
hOAT2(-100/+28)	AGACAGGTAGAGTCCCTGGAGAC	CCTCAAAGCCCCATGCTGCTCACCCA
mOat2(-1100/+89)	CCGCTCGAGAATTTTCTCAGAAATGCAAA	CCCAAGCTTAGCCACCCACCTTGTGCAGCA
mOat2(-400/+89)	CCGCTCGAGTGAGTCAAACCTGTGTGTGCTT	CCCAAGCTTAGCCACCCACCTTGTGCAGCA
mOat2(-150/+89)	CCGCTCGAGGGAATGGTGGCCTTTCCCTTAG	CCCAAGCTTAGCCACCCACCTTGTGCAGCA
EMSA		
Cyp7a1	GGACTTAGTTCAAGGCCAGATAATGCT	AGCATTATCTGGCCTTGAAGTAACTCC
Oat2	TCTTCCGAAGGCCCTGAGTTCATTCC	GGAATGGAACCTAGGGCCTTCGGAAGA
Oat2(mutant)	TCTTCCACCTGTATGCGACCGCATTCC	GGAATGCGGTGCGATACAGGTGGAAGA
CHIP		
Cyp7a1	GCTTATCGACTATTGCAGCTCTCT	CTGGCCTTGAAGTAACTGCTCATCT
Oat2	AAAGAGGCAGACTGGACT	GTTGCTGGGAATGGAAGT
siRNA		
siLrh-1-1	GCAUGGACUUACAGCUAUUTT	AAUAGCUGUAAGUCCAUGCTT
siLrh-1-2	CCUUGUCAUGCUGCCCAAATT	UUUGGGCAGCAUGACAAGGTT
siLrh-1-3	GCAGAAGAAAGCCCUCAUUTT	AAUGAGGCUUUCUUCUGCTT
siLrh-1-4	CCAACCCUAUGGUACAUUUTT	AAAGUGACCAUAGGGUUGGTT
siLRH-1	AAGGATCCATCTTCTGGTTA	TAACCAGGAAGATGGATCCTT

Quantification of PGE₂ and Docetaxel. PGE₂ and docetaxel were quantified using an UPLC-QTOF/MS system (Waters) and a BEH C18 column (2.1 × 50 mm, 2.6 μm; Waters). The mobile phase was 0.1% formic acid (mobile phase A) and 0.1% formic acid in acetonitrile (mobile phase B). The flow rate was set at 0.3 ml/min. The gradient elution program was 10% B at 0–1 minute, 10%–95% B at 1–4.5 minutes, and 95%–10% B at 4.5–5 minutes. Mass spectrometer was operated at the negative ion full scan mode for PGE₂, whereas docetaxel was analyzed at the positive ion mode. The capillary, sampling cone, and extraction cone voltages were set at 3000, 25, and 4 V, respectively. The source and desolvation temperatures were 120 and 400°C, respectively. Peak areas of PGE₂ and docetaxel were recorded with extract masses of m/z 351.22 ± 0.05 and 808.33 ± 0.05 Da, respectively. The calibration curves for PGE₂ and docetaxel were linear ($r^2 > 0.999$) over the entire concentration range (i.e., 3–1000 ng/ml for PGE₂ and 10–2000 ng/ml for docetaxel). The limit of detection (defined as a signal/noise ratio of 3:1) was 30 pg/ml for both PGE₂ and docetaxel, and the limit of quantitation (defined as a signal/noise ratio of 10:1) was 100 pg/ml for both compounds. Interday/intraday variabilities of PGE₂ and docetaxel were less than 5% and 3%, respectively.

Statistical Analysis. Data are presented as mean ± S.D. Pharmacokinetic parameters were analyzed for statistical significance using the normal hypothesis test (z -test). All other data were analyzed for statistical significance using Student's t test. The level of significance was set at $P < 0.05$.

Results

Lrh-1/LRH-1 Regulates Oat2/OAT2 Expression in Hepatoma Cells. We first generated an expression vector carrying mouse full-length Lrh-1. Transfection of this vector into mouse hepatoma Hepa1-6 cells led to overexpression of Lrh-1 (a 144-fold increase in mRNA and a 2.8-fold increase in protein level) (Fig. 1, A and B). Interestingly, overexpression of Lrh-1 resulted in a marked increase (2.2-fold) in Oat2 mRNA (Fig. 1A). Accordingly, the Oat2 protein level was significantly increased (1.8-fold) (Fig. 1B).

Next, we tried to examine the effects of Lrh-1 downregulation on Oat2 expression. Four different siRNA fragments (i.e. siLrh-1-1, siLrh-1-2, siLrh-1-3, siLrh-1-4) were designed and synthesized for Lrh-1 (Table 1). Of four siRNAs, siLrh-1-3 was the most efficient one in silencing Lrh-1 (Fig. 1C) and thus were used for further experiments. Use of this siRNA caused an effective downregulation of Lrh-1 expression (an 80% reduction in mRNA and a 47% decrease in protein level) (Fig. 1, D and E). Consistent with its activation effect on Oat2, Lrh-1 knockdown led a significant reduction in Oat2 mRNA (62%) and protein level (55%) (Fig. 1, D and E). In addition, silencing of Lrh-1 led to markedly reduced uptake of PGE₂, a prototypical endogenous substrate of Oat2, into Hepa1-6 cells (Fig. 1F).

Furthermore, we assessed the regulatory potential of human LRH-1 on OAT2 expression using HepG2 cells. Overexpression of full-length LRH-1 led to a significant increase in mRNA level of OAT2 (Fig. 2A). Consistently, mRNA expression of OAT2 was significantly decreased in LRH-1 knockdown cells (Fig. 2B). Taken together, these data suggested that Lrh-1/LRH-1 was a positive regulator of Oat2/OAT2 expression.

Conditional Deletion of Hepatic Lrh-1 Downregulates Oat2 in Mice. We showed relatively high expression of Lrh-1 protein in the livers of wild-type mice (Fig. 3A). We further generated liver-specific Lrh-1 knockout mice (named Lrh-1^{hep-/-} mice) by intercrossing Lrh-1 floxed and Alb-Cre mice (Fig. 3B). PCR analysis of genomic DNA from the tails of Lrh-1^{hep-/-} mice showed recombination of loxP sites and Alb-Cre recombinase (Fig. 3B). We also confirmed the Lrh-1 was absent in the genetic mice (Fig. 3C). Hepatic deletion of Lrh-1 led to significant decreases in Oat2 mRNA and the mRNA level of Cyp8b1, a known LRH-1 target gene (Sablin et al., 2003) (Fig. 3C). Consistent with the mRNA change, the Oat2 protein was markedly reduced in the liver (Fig. 3D).

Fig. 1. Lrh-1 regulates Oat2 expression and activity in Hepa1-6 cells. Hepa1-6 cells were seeded onto six-well plates for 12 hours and then transfected with 2 μ g Lrh-1 expression plasmid or 50 nM siLrh-1. After 24-hour transfection, Oat2 mRNA and protein were quantified by qPCR and Western blotting, respectively. (A) Effects of Lrh-1 overexpression on Oat2 mRNA. (B) Effects of Lrh-1 overexpression on Oat2 protein. (C) Lrh-1 gene silencing efficiency of four siRNAs. (D) Effects of Lrh-1 knockdown on Oat2 mRNA. (E) Effects of Lrh-1 knockdown on Oat2 protein. (F) Effects of Lrh-1 knockdown on cellular uptake of PGE₂ at different time points. After 36-hour transfection of siLrh-1, the cells were incubated with Hanks' balanced salt solution (HBSS) containing 5 μ M PGE₂ for 0.5, 1, or 2 hours. Intracellular levels of PGE₂ were quantified by UPLC-QTOF/MS. Data are presented as mean $\pm$ S.D. ($n = 6$). * $P < 0.05$ (t test).

Lrh-1/LRH-1 is a Transcriptional Activator of Oat2/OAT2. An Oat2 luciferase reporter (with -2.0 -kb proximal promoter) was constructed to investigate a possible role of Lrh-1 in transcriptional regulation of Oat2. We observed an activation effect of Lrh-1 on Oat2 transcription (Fig. 4A). The extent of activation was positively correlated with the transfected amounts of Lrh-1 (Fig. 4A). By contrast, knockdown of Lrh-1 resulted in a decreased promoter activity (Fig. 4A). These results suggested that Lrh-1 was a transcriptional activator of Oat2.

Sequence analysis of Oat2 promoter using JASPAR algorithm (jaspar.genereg.net) revealed four potential Lrh-1 binding sites (i.e.,

A-, B-, C-, and D-sites in Fig. 4B). Accordingly, four shorter (truncated) promoter constructs (i.e., -1.1 , -0.4 , -0.15 and 0.03 kb) with the deletion of one or more potential binding sites were generated (Fig. 4B). Lrh-1 greatly enhanced the transcriptional activities (a seven to eightfold increase) of both -2.0 - and -1.1 -kb constructs (Fig. 4B). However, transcriptional activation by Lrh-1 was diminished for other promoter constructs (i.e., -0.4 , -0.15 and 0.02 kb) (Fig. 4B). The data suggested that the core regulatory element responsible for Oat2 promoter activity was located between -1.1 and -0.4 kb (i.e., the B-site) (Fig. 4B). In a similar manner, an LRH-1 response element (at position -746) within the human OAT2 promoter was identified (Fig. 4C).

Fig. 2. LRH-1 regulates OAT2 expression in HepG2 cells. (A) Effects of LRH-1 overexpression on OAT2 mRNA. HepG2 cells were seeded onto six-well plates for 12 hours and then transfected with 2 μ g LRH-1 expression vector or equal amount of pcDNA3.1 vector. After 24-hour transfection, OAT2 mRNA was quantified by qPCR from total RNA. (B) Effects of LRH-1 knockdown on OAT2 mRNA. HepG2 cells were seeded onto six-well plates for 12 hours and then transfected with 50 nM siLRH-1 or equal amount of siRNA for negative control (siNC). After 24-hour transfection, OAT2 mRNA was quantified by qPCR from total RNA. Data are presented as mean $\pm$ S.D. ($n = 6$). * $P < 0.05$ (t test).

Fig. 3. Hepatic deletion of *Lrh-1* downregulates *Oat2* expression in mice. (A) Relative protein levels of *Lrh-1* in various tissues of wild-type (*Lrh-1^{flox/flox}*) mice. (B) PCR analysis of genomic DNA from the tails of wild-type (WT) and *Lrh-1^{hep-/-}* mice. The sizes of PCR products corresponding the flox and Alb-Cre are indicated. (C) qPCR analysis of *Oat2* in the livers from WT and *Lrh-1^{hep-/-}* mice. (D) Western blotting of *Oat2* in the livers from WT and *Lrh-1^{hep-/-}* mice. Data are presented as mean $\pm$ S.D. ($n = 5$). * $P < 0.05$ (t test).

Next, EMSA assay was performed to confirm binding of *Lrh-1* to the B-site (−716/−702 bp) using a biotin-labeled dimerized oligonucleotide (i.e., −722 to −696 bp of *Oat2* promoter). The *Lrh-1* binding site of *Cyp7a1* (*Cyp7a1*-*LrhRE*, the sequence is provided in Table 1) was used in a control experiment. As expected, *Cyp7a1*-*LrhRE* generated a DNA-protein complex that was diminished upon addition of unlabeled probe (Fig. 5A). Interestingly, the *Oat2* probe was able to form a complex with *Lrh-1* protein (Fig. 5A). Formation of this complex was inhibited by unlabeled probe but unaffected in the presence of mutated probe (the sequence is provided in Table 1) (Fig. 5A). The results strongly indicated that *Lrh-1* protein bound directly to the B-site of *Oat2* promoter. To confirm the interaction of *Lrh-1* with *Oat2* promoter in vivo, ChIP assays were performed using mouse liver samples. We observed significant recruitment of *Lrh-1* to the B-site (Fig. 5B). Overall, these data indicated that *Lrh-1* activated the transcription of *Oat2* through its specific binding to the B-site (i.e., the −716- to −702-bp region).

***Lrh-1* Knockdown Led to Reduced Uptake of Docetaxel to Hepa1-6 Cells.** Docetaxel is a known substrate of *Oat2* (Baker et al., 2009). Knockdown of *Lrh-1* by siRNA led to a significant decrease in uptake of docetaxel (20 μ M) into Hepa1-6 cells over a 2-hour period (Fig. 6A). Reduced cellular uptake of docetaxel was also observed at a wide range of dosing concentrations (10–80 μ M) (Fig. 6B).

Hepatic Deletion of *Lrh-1* Alters Docetaxel Pharmacokinetics in Mice. Pharmacokinetic studies were performed with wild-type (*Lrh-1^{fl/fl}*) and *Lrh-1^{hep-/-}* mice after intraperitoneal injection of docetaxel (10 mg/kg). The plasma docetaxel concentration versus time curve was significantly altered in *Lrh-1^{hep-/-}* mice (Fig. 7A). *Lrh-1^{hep-/-}* mice showed increased C_{max} and AUC (i.e., the area under the curve, representing the systemic exposure of docetaxel) values (Table 2). By contrast, the liver docetaxel concentrations were lowered in *Lrh-1^{hep-/-}* mice at the time points of ≤ 2 hours (Fig. 7B). Accordingly, the AUC value for the liver concentration-time profile was significantly decreased (Table 2). These

data indicated that *Lrh-1* regulated docetaxel pharmacokinetics via modulation of *Oat2*-mediated hepatic uptake of the drug (Fig. 7C).

Discussion

This study for the first time demonstrated that the nuclear receptor *Lrh-1*/LRH-1 regulated *Oat2*/OAT2 expression in mice in vivo and in mouse and human hepatoma cells. Through a combination of luciferase reporter, mobility shift, and ChIP assays, it was shown that *Lrh-1*/LRH-1 transactivated *Oat2*/OAT2 through its direct binding to an *Lrh-1*/LRH-1 response element (−716 to −702 bp for mice and −746 to −732 bp for humans) within the gene promoter (Figs. 4 and 5). Additionally, genetic deletion of hepatic *Lrh-1* altered the pharmacokinetics of docetaxel, an *Oat2* substrate, in mice. Alteration in docetaxel pharmacokinetics resulted from reduced drug uptake to the liver due to downregulated *Oat2* expression as the expressions of *Oatp* transporters (also participate in hepatic uptake of docetaxel; Sprowl and Sparreboom, 2014) were unaffected (Supplemental Fig. 1). Collectively, *Lrh-1* transcriptionally regulated *Oat2*, thereby impacting tissue uptake and pharmacokinetics of *Oat2* substrates (Fig. 7C).

In addition to the chemotherapeutic agents such as docetaxel, the OAT2 transporter participates in the excretion of many other types of drugs including nonsteroidal anti-inflammatory drugs, angiotensin-converting enzyme inhibitors, and antibiotics (Russel et al., 2002; Cutler and Choo, 2011; Marada et al., 2015). Genetic deficiency of OAT2 has been linked to the alterations (e.g., reduced urinary excretion and higher level of circulating drug) in disposition of these drugs (Russel et al., 2002). Therefore, identification of LRH-1 as a novel pharmacokinetic determinant of OAT2 substrates assume great importance because this knowledge will facilitate a better understanding of varied pharmacokinetics and possibly pharmacodynamics of OAT2 substrates.

Fig. 4. Lrh-1/LRH-1 is a transcriptional activator of Oat2/OAT2. HEK293T cells were cotransfected with Lrh-1 expression plasmid or with siLrh-1 for 24 hours. (A) Effects of Lrh-1 overexpression or knockdown on transcriptional activity of Oat2 promoter. (B) Lrh-1 stimulates mouse Oat2 promoter activity by binding to the $-716/-702$ region (B-site). (C) LRH-1 stimulates human OAT2 promoter activity by binding to the $-746/-732$ region (B-site). Potential Lrh-1/LRH-1 binding elements in Oat2/OAT2 promoter region were predicted from JASPAR algorithm (jaspar.genereg.net). Capitals in squares indicate the putative binding motifs for Lrh-1/LRH-1. Data are mean $\pm$ S.D. ($n = 6$). $*P < 0.05$ (t test).

The finding that Oat2 was transcriptionally activated by Lrh-1 was consistent with previous microarray data that show hepatic Oat2 is markedly reduced in Lrh-1-deficient mice (Stein et al., 2014). Moreover,

expression control of an uptake transporter such as Oat2 by Lrh-1 suggested an important role of this nuclear receptor in drug absorption and disposition. This was evidenced by the fact that downregulated

Fig. 5. Interactions of Lrh-1 with its response element (B-site) in the Oat2 promoter. (A) EMSA assay showing formation of a DNA-protein complex between Lrh-1 and the Oat2 B site. EMSA was performed with labeled Oat2 probe or labeled Cyp7a1 probe in the presence of nuclear extracts from Hepa1-6 cells. Competitive EMSAs on labeled Oat2 probe and labeled Cyp7a1 probe were performed by adding 50-fold molar excess of unlabeled Oat2 or Cyp7a1 oligonucleotides. (B) ChIP assay showing recruitment of Lrh-1 to Oat2 promoter. ChIP assay was performed using an anti-LRH-1 antibody, and qPCR was performed using primers specific for Oat2 or Cyp7a1. The primer sequences for the ChIP-PCR are provided in Table 1. Data are mean $\pm$ S.D. ($n = 6$). $*P < 0.05$ (t test).

Fig. 6. Lrh-1 knockdown led to reduced uptake of docetaxel into Hepa1-6 cells. Hepa1-6 cells were seeded onto six-well plates for 12 hours, and then transfected with 50 nM siLrh-1 or equal amount of siNC. After 36-hour transfection, the cells were incubated with docetaxel. (A) Effects of Lrh-1 knockdown on uptake of docetaxel at different time points. (B) Effects of Lrh-1 knockdown on uptake of docetaxel with different dosing concentrations. Data are mean $\pm$ S.D. ($n = 6$). $*P < 0.05$ (t test).

Lrh-1 was associated with decreased cellular uptake of PGE2 and docetaxel as well as altered docetaxel pharmacokinetics (Fig. 1F; Figs. 6 and 7). Therefore, Lrh-1 expression may be a contributing factor to individual variations in pharmacokinetics/efficacy of Oat2 substrates. On the other hand, a growing number of Lrh-1 ligands are being identified (Lee et al., 2011; Whitby et al., 2011; Benod et al., 2013). Ligand modulation of LRH-1 activity would also alter the transport and disposition of OAT2 substrates, thereby accounting for drug-drug interactions.

Our data suggested a potential role of LRH-1 in maintenance of body homeostasis because its target gene OAT2 is known to transport many endogenous substances (e.g., glutamate, uric acid, creatinine, and PGE2) with important physiologic functions (Shen et al., 2015; Xu et al., 2016). This is also supported by the fact that Lrh-1^{hep-/-} mice show a marked

decrease in hepatic glutamate content compared with Lrh-1^{hep+/+} mice (Xu et al., 2016). Therefore, LRH-1 may be targeted to modulate the levels of endogenous substances for prevention and treatment of certain diseases. For instance, modulation of LRH-1 activity may change the glutamate level that would be exploited to alleviate glutamate excitotoxicity in the central nervous system. Also, the serum level of uric acid may be modified via LRH-1 activation to manage various diseases such as gout, hypertension, and cardiovascular diseases (Sato et al., 2010).

LRH-1 has been reported to serve as a competence factor that enhances the regulatory effects of other nuclear receptors (Lu et al., 2000; Luo et al., 2001). We tried to determine whether Lrh-1 acts as a competence factor for Hnf4 α , because HNF4 α is a known transcriptional activator of OAT2 (Popowski et al., 2005). Interestingly, Lrh-1 and Hnf4 α showed an additive effect in the induction of Oat2 promoter

Fig. 7. Hepatic deletion of Lrh-1 alters docetaxel pharmacokinetics in mice. (A) Plasma concentrations of docetaxel in wild-type (Lrh-1^{flx/flx}) and Lrh-1^{hep-/-} mice at different time points after docetaxel treatment (10 mg/kg, i.p., $n = 6$). (B) Liver concentrations of docetaxel in Lrh-1^{flx/flx} and Lrh-1^{hep-/-} mice at different time points after docetaxel treatment (10 mg/kg, i.p., $n = 6$). (C) Proposed regulatory model elucidating a critical role of LRH-1 in regulation of OAT2 expression and docetaxel pharmacokinetics.

TABLE 2

Pharmacokinetic parameters for the docetaxel concentration-time profiles

Parameter	Unit	WT	Lrh-1 ^{hep-/-}
Plasma			
AUC	μg/ml·h	4.75 ± 0.21	6.81 ± 0.32*
MRT	h	1.86 ± 0.09	1.42 ± 0.02*
V _{ss}	l/kg	3.07 ± 0.44	1.79 ± 0.28*
CL	l/h per kilogram	2.13 ± 0.30	1.41 ± 0.05*
Liver			
AUC	μg/ml·h	7.61 ± 0.22	5.98 ± 1.01*

*P < 0.05 vs. WT group. WT, wild type (Lrh-1^{fl/fl}).

activity (Supplemental Fig. 2), suggesting independent activation of Oat2 transcription. On the other hand, the binding site in OAT2 promoter for LRH-1 is located at -746/-733 bp, whereas HNF4α binds to a DR-1 site (-329/-317 bp). As for Oat2, the Lrh-1 binding site does not occur in conjunction with Hnf4α binding site, supporting independent regulation of Oat2 by Lrh-1 and Hnf4α. Therefore, in addition to acting as a competence factor, LRH-1 can independently regulate gene expression.

It was noteworthy that we were unable to determine the kinetic parameters for uptake transport of docetaxel into Hepal-6 cells (Fig. 6B). This was because the rates of transport were nearly linear to the test concentrations (10–80 μM). Attempts to obtaining transport rates at high concentrations of more than 100 μM failed due to limited aqueous drug solubility (about 100 μM). Nevertheless, pairwise comparisons of cellular transport at different time points and different drug concentrations consistently indicated Lrh-1-dependent uptake of docetaxel (Fig. 6).

This study focused on the assessment of the impact of hepatic Lrh-1 on drug pharmacokinetics because this protein is abundantly expressed in the liver and the liver is the major drug-eliminating organ (Fig. 3A). However, since both Lrh-1 and Oat2 is also expressed in the kidney and intestine, the other two major drug-eliminating organs (Fig. 3) (Kusuhara and Sugiyama, 2002), there is a high possibility that Lrh-1 will have impact on renal and/or intestinal disposition of Oat2 substrates. Nevertheless, whether renal and intestinal Lrh-1 regulate Oat2 expression and pharmacokinetics warrants further investigations.

We provided in vitro and in vivo evidence that mouse Lrh-1 transactivates hepatic Oat2 to alter drug pharmacokinetics. By contrast, relatively limited data (i.e., those generated from HepG2 cells and luciferase promoter assays; Figs. 2 and 4) are available here supporting the regulation of human OAT2 by LRH-1. It was acknowledged that the specific DNA region (-746 to -732-bp) for LRH-1 binding to human OAT2 needs a further validation with mobility shift and/or ChIP assays (Fig. 4). Nevertheless, the ultimate question whether LRH-1 regulates OAT2 and drug pharmacokinetics in humans as its counterpart did in mice awaits further explorations.

In summary, Lrh-1/LRH-1 upregulated Oat2/OAT2 at the mRNA, protein, and activity levels in hepatoma cells. Consistently, hepatic deletion of Lrh-1 downregulated Oat2 expression and hepatic docetaxel uptake, but increased the system exposure of docetaxel. Through a combination of luciferase reporter, mobility shift, and ChIP assays, we showed that Lrh-1 transactivated Oat2 by its specific binding to -716/-702-bp promoter region. Taken together, Lrh-1 transcriptionally regulates Oat2, thereby impacting tissue uptake and pharmacokinetics of Oat2 substrates.

Authorship Contributions

Participated in research design: Yu, Zhang, Wu.

Conducted experiments: Yu, Zhang, Guo.

Performed data analysis: Yu, Zhang, Wu.

Wrote or contributed to the writing of the manuscript: Yu, Wu.

References

- Anzai N and Endou H (2007) Drug discovery for hyperuricemia. *Expert Opin Drug Discov* 2: 1251–1261.
- Anzai N, Kanai Y, and Endou H (2006) Organic anion transporter family: current knowledge. *J Pharmacol Sci* 100:411–426.
- Baker SD, Sparreboom A, and Verweij J (2006) Clinical pharmacokinetics of docetaxel: recent developments. *Clin Pharmacokinet* 45:235–252.
- Baker SD, Verweij J, Cusatis GA, van Schaik RH, Marsh S, Orwick SJ, Franke RM, Hu S, Schuetz EG, Lamba V, et al. (2009) Pharmacogenetic pathway analysis of docetaxel elimination. *Clin Pharmacol Ther* 85:155–163.
- Benod C, Carlsson J, Uthayaruban R, Hwang P, Irwin JJ, Doak AK, Shoichet BK, Sablin EP, and Fletterick RJ (2013) Structure-based discovery of antagonists of nuclear receptor LRH-1. *J Biol Chem* 288:19830–19844.
- Burger H, Loos WJ, Eechoute K, Verweij J, Mathijssen RHJ, and Wiemer EAC (2011) Drug transporters of platinum-based anticancer agents and their clinical significance. *Drug Resist Updat* 14:22–34.
- Chand AL, Herridge KA, Howard TL, Simpson ER, and Clyne CD (2011) Tissue-specific regulation of aromatase promoter II by the orphan nuclear receptor LRH-1 in breast adipose stromal fibroblasts. *Steroids* 76:741–744.
- Cheng Y, Vapurcuyan A, Shahidullah M, Aleksunes LM, and Pelis RM (2012) Expression of organic anion transporter 2 in the human kidney and its potential role in the tubular secretion of guanine-containing antiviral drugs. *Drug Metab Dispos* 40:617–624.
- Connolly RM, Rudek MA, Garrett-Mayer E, Jeter SC, Donehower MG, Wright LA, Zhao M, Fetting JH, Emens LA, Stearns V, et al. (2011) Docetaxel metabolism is not altered by imatinib: findings from an early phase study in metastatic breast cancer. *Breast Cancer Res Treat* 127: 153–162.
- Cutler MJ and Choo EF (2011) Overview of SLC22A and SLC22B families of drug uptake transporters in the context of cancer treatments. *Curr Drug Metab* 12:793–807.
- de Graan AJ, Lancaster CS, Obaidat A, Hagenbuch B, Elens L, Friberg LE, de Bruijn P, Hu S, Gibson AA, Bruun GH, et al. (2012) Influence of polymorphic OATP1B-type carriers on the disposition of docetaxel. *Clin Cancer Res* 18:4433–4440.
- del Castillo-Olivares A and Gil G (2000) Alpha 1-fetoprotein transcription factor is required for the expression of sterol 12alpha-hydroxylase, the specific enzyme for cholic acid synthesis. Potential role in the bile acid-mediated regulation of gene transcription. *J Biol Chem* 275: 17793–17799.
- Ehrlichova M, Vaclavikova R, Ojima I, Pepe A, Kuznetsova LV, Chen J, Truksa J, Kovar J, and Gut I (2005) Transport and cytotoxicity of paclitaxel, docetaxel, and novel taxanes in human breast cancer cells. *Naunyn-Schmiedeberg Arch Pharmacol* 372:95–105.
- Fayard E, Auwerx J, and Schoonjans K (2004) LRH-1: an orphan nuclear receptor involved in development, metabolism and steroidogenesis. *Trends Cell Biol* 14:250–260.
- Franke RM, Carducci MA, Rudek MA, Baker SD, and Sparreboom A (2010) Castration-dependent pharmacokinetics of docetaxel in patients with prostate cancer. *J Clin Oncol* 28:4562–4567.
- Freeman LA, Kennedy A, Wu J, Bark S, Remaley AT, Santamarina-Fojo S, and Brewer HB Jr (2004) The orphan nuclear receptor LRH-1 activates the ABCG5/ABCG8 intergenic promoter. *J Lipid Res* 45:1197–1206.
- Hopper-Borge E, Chen ZS, Shchavaleva I, Belinsky MG, and Kruh GD (2004) Analysis of the drug resistance profile of multidrug resistance protein 7 (ABCC10): resistance to docetaxel. *Cancer Res* 64:4927–4930.
- Jigorel E, Le Vee M, Boursier-Neyret C, Parmentier Y, and Fardel O (2006) Differential regulation of sinusoidal and canalicular hepatic drug transporter expression by xenobiotics activating drug-sensing receptors in primary human hepatocytes. *Drug Metab Dispos* 34:1756–1763.
- Kobayashi Y, Ohshiro N, Sakai R, Ohbayashi M, Kohyama N, and Yamamoto T (2005a) Transport mechanism and substrate specificity of human organic anion transporter 2 (hOat2 [SLC22A7]). *J Pharm Pharmacol* 57:573–578.
- Kobayashi Y, Sakai R, Ohshiro N, Ohbayashi M, Kohyama N, and Yamamoto T (2005b) Possible involvement of organic anion transporter 2 on the interaction of theophylline with erythromycin in the human liver. *Drug Metab Dispos* 33:619–622.
- Kusuhara H and Sugiyama Y (2002) Role of transporters in the tissue-selective distribution and elimination of drugs: transporters in the liver, small intestine, brain and kidney. *J Control Release* 78:43–54.
- Lee JM, Lee YK, Mamros JL, Busby SA, Griffin PR, Pathak MC, Ortlund EA, and Moore DD (2011) A nuclear-receptor-dependent phosphatidylcholine pathway with antidiabetic effects. *Nature* 474:506–510.
- Lu D, Wang S, Xie Q, Guo L, and Wu B (2017) Transcriptional regulation of human UDP-glucuronosyltransferase 2B10 by farnesoid X receptor in human hepatoma HepG2 cells. *Mol Pharm* 14:2899–2907.
- Lu TT, Makishima M, Repp J, Schoonjans K, Kerr TA, Auwerx J, and Mangelsdorf DJ (2000) Molecular basis for feedback regulation of bile acid synthesis by nuclear receptors. *Mol Cell* 6:507–515.
- Luo Y, Liang CP, and Tall AR (2001) The orphan nuclear receptor LRH-1 potentiates the sterol-mediated induction of the human CETP gene by liver X receptor. *J Biol Chem* 276:24773.
- Maher JM, Slitt AL, Callaghan TN, Cheng X, Cheung C, Gonzalez FJ, and Klaassen CD (2006) Alterations in transporter expression in liver, kidney, and duodenum after targeted disruption of the transcription factor HNF1alpha. *Biochem Pharmacol* 72:512–522.
- Marada VV, Flörl S, Kühne A, Müller J, Burckhardt G, and Hagos Y (2015) Interaction of human organic anion transporter 2 (OAT2) and sodium taurocholate cotransporting polypeptide (NTCP) with antineoplastic drugs. *Pharmacol Res* 91:78–87.
- Nishino S, Itoh A, Matsuoka H, Maeda K, and Kamoshida S (2013) Immunohistochemical analysis of organic anion transporter 2 and reduced folate carrier 1 in colorectal cancer: significance as a predictor of response to oral uracil/fluorouracil plus leucovorin chemotherapy. *Mol Clin Oncol* 1:661–667.
- Popowski K, Eloranta JJ, Saborowski M, Fried M, Meier PJ, and Kullak-Ublick GA (2005) The human organic anion transporter 2 gene is transactivated by hepatocyte nuclear factor-4 alpha and suppressed by bile acids. *Mol Pharmacol* 67:1629–1638.

- Russel FGM, Masereeuw R, and van Aubel RAMH (2002) Molecular aspects of renal anionic drug transport. *Annu Rev Physiol* **64**:563–594.
- Sablin EP, Krylova IN, Fletterick RJ, and Ingraham HA (2003) Structural basis for ligand-independent activation of the orphan nuclear receptor LRH-1. *Mol Cell* **11**:1575–1585.
- Sato M, Mamada H, Anzai N, Shirasaka Y, Nakanishi T, and Tamai I (2010) Renal secretion of uric acid by organic anion transporter 2 (OAT2/SLC22A7) in human. *Biol Pharm Bull* **33**: 498–503.
- Sekine T, Cha SH, Tsuda M, Apiwattanakul N, Nakajima N, Kanai Y, and Endou H (1998) Identification of multispecific organic anion transporter 2 expressed predominantly in the liver. *FEBS Lett* **429**:179–182.
- Shen H, Liu T, Morse BL, Zhao Y, Zhang Y, Qiu X, Chen C, Lewin AC, Wang XT, Liu G, et al. (2015) Characterization of organic anion transporter 2 (SLC22A7): a highly efficient transporter for creatinine and species-dependent renal tubular expression. *Drug Metab Dispos* **43**:984–993.
- Shirakawa K, Takara K, Tanigawara Y, Aoyama N, Kasuga M, Komada F, Sakaeda T, and Okumura K (1999) Interaction of docetaxel (“taxotere”) with human P-glycoprotein. *Jpn J Cancer Res* **90**:1380–1386.
- Sprowl JA and Sparreboom A (2014) Uptake carriers and oncology drug safety. *Drug Metab Dispos* **42**:611–622.
- Stein S, Oosterveer MH, Matakis C, Xu P, Lemos V, Havinga R, Dittner C, Ryu D, Menzies KJ, Wang X, et al. (2014) SUMOylation-dependent LRH-1/PROX1 interaction promotes atherosclerosis by decreasing hepatic reverse cholesterol transport. *Cell Metab* **20**:603–613.
- Sun W, Wu RR, van Poelje PD, and Erion MD (2001) Isolation of a family of organic anion transporters from human liver and kidney. *Biochem Biophys Res Commun* **283**:417–422.
- Whitby RJ, Stec J, Blind RD, Dixon S, Leesnitzer LM, Orband-Miller LA, Williams SP, Willson TM, Xu R, Zuercher WJ, et al. (2011) Small molecule agonists of the orphan nuclear receptors steroidogenic factor-1 (SF-1, NR5A1) and liver receptor homologue-1 (LRH-1, NR5A2). *J Med Chem* **54**:2266–2281.
- Xu P, Oosterveer MH, Stein S, Demagry H, Ryu D, Moullan N, Wang X, Can E, Zamboni N, Comment A, et al. (2016) LRH-1-dependent programming of mitochondrial glutamine processing drives liver cancer. *Genes Dev* **30**:1255–1260.
- Zhang T, Zhao M, Lu D, Wang S, Yu F, Guo L, Wen S, and Wu B (2018) REV-ERB α regulates CYP7A1 through repression of liver receptor homolog-1. *Drug Metab Dispos* **46**:248–258.
- Zhou L, Naraharisetti SB, Wang H, Unadkat JD, Hebert MF, and Mao Q (2008) The breast cancer resistance protein (Bcrp1/Abcg2) limits fetal distribution of glyburide in the pregnant mouse: an obstetric-fetal pharmacology research unit network and University of Washington specialized center of research study. *Mol Pharmacol* **73**:949–959.

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