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Transcriptional Regulation of Human Cytosolic Sulfotransferase 1C3 by Peroxisome Proliferator-Activated Receptor γ in LS180 Human Colorectal Adenocarcinoma Cells

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ABSTRACT

Cytosolic sulfotransferase 1C3 (SULT1C3) is the least characterized of the three human SULT1C subfamily members. Originally identified as an orphan SULT by computational analysis of the human genome, we recently reported that SULT1C3 is expressed in human intestine and LS180 colorectal adenocarcinoma cells and is upregulated by agonists of peroxisome proliferator-activated receptor (PPAR) α and γ. To determine the mechanism responsible for PPAR-mediated upregulation, we prepared reporter plasmids containing fragments of the SULT1C3 5′-flanking region. During initial attempts to amplify a 2.8-kb fragment from different sources of human genomic DNA, a 1.9-kb fragment was sometimes coamplified with the expected 2.8-kb fragment. Comparison of the 1.9-kb fragment sequence to the published SULT1C3 5′-flanking sequence revealed an 863-nt deletion (nt −146 to −1008 relative to the transcription start site). Transfection analysis in LS180 cells demonstrated that PPARα, δ, and γ agonist treatments induced luciferase expression from a reporter plasmid containing the 2.8-kb but not the 1.9-kb fragment. The PPAR agonists also activated a 1-kb reporter containing the 863-nt deletion region. Computational analysis identified three peroxisome proliferator response elements (PPREs) within the 863-nt region and serial deletions and site-directed mutations indicated that the most distal PPRE (at nt −769) was essential for obtaining PPAR-mediated transcriptional activation. Although agonists of all three PPARs could activate SULT1C3 transcription, RNA interference analysis indicated the predominance of PPARγ. These data demonstrate that the PPARγ regulatory network includes SULT1C3 and imply that this enzyme contributes to the control of such PPARγ-regulated intestinal processes as growth, differentiation, and metabolism.

Introduction

The cytosolic sulfotransferases (SULTs) are conjugating enzymes that catalyze the transfer of a sulfonate group (SO_3^-) from 3′-phosphoadenosine 5′-phosphosulfate to numerous alcohol, phenol, amine, N-oxide, and N-hydroxy substrates. SULTs regulate various physiologic processes by sulfonating endogenous molecules, including hormones, bile acids, and neurotransmitters such as dopamine. SULTs also play important roles in the detoxification of xenobiotics but can also contribute to the bioactivation of some procarcinogens (Yamazoe et al., 1995; Zhang et al., 2012).

The human genome contains 13 SULT genes that are classified into four families (Blanchard et al., 2004). Of these, the SULT1 (phenol sulfotransferases) and SULT2 families (hydroxysteroid sulfotansferases) have been best characterized and are further divided into subfamilies (Dooley et al., 2000; Glatt et al., 2001). The human SULT1C subfamily includes three members, SULT1C2 (previously known as SULT1C1), SULT1C3, and SULT1C4 (previously known as SULT1C2), which are located in a cluster on chromosome 2 (Freimuth et al., 2000, 2004; Blanchard et al., 2004). Human SULT1C2 has been detected in fetal and adult kidney, liver, and small intestine, as well as adult stomach (Her et al., 1997; Teubner et al., 2007), whereas human SULT1C4 has been detected in fetal lung, kidney, and heart, as well as in adult kidney and in colon cancer cells (Sakakibara et al., 1998; Meinl et al., 2008b). SULT1C3 is a relatively uncharacterized (orphan) enzyme that was predicted through computational analysis of the human genome (Freimuth et al., 2004). The predicted SULT1C3 gene has a duplication of exons 7 and 8 and theoretically could be transcribed and processed into

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ABBREVIATIONS: AhR, aryl hydrocarbon receptor; DMSO, dimethyl sulfoxide; FXR, farnesoid X receptor; GW0742, 4-[[[2-[3-fluoro-4-(trifluoromethyl)phenyl]-4-methyl-5-thiazolyl]methyl]thio]-2-methylphenoxyacetic acid; GW3965, 3-[3-[[[2-chloro-3-(trifluoromethyl)phenyl]methyl](2,2 diphenylethyl)amino]propoxy]benzeneacetic acid hydrochloride; GW4064, 3-[2-[2-chloro-4-[[3-(2,6-dichlorophenyl)-5-(1-methylethyl)-4-isoxazolyl]methoxy]phenyl]ethenyl]benzoic acid; GW7647, 2-[[4-[2-[[(cyclohexylamino)carbonyl](4-cyclohexylbutyl)amino]ethyl]phenyl]thio]-2-methylpropanoic acid; LXR, liver X receptor; MEM, minimum essential medium; PAPS, 3′-phosphoadenosine-5′-phosphosulfate; PCR, polymerase chain reaction; PPAR, peroxisome proliferator-activated receptor; PPRE, peroxisome proliferator response element; PXR, pregnane X receptor; RACE, rapid amplification of cDNA ends; siRNA, small interfering RNA; SULT, cytosolic sulfotransferase; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin.

four splice variants containing exons 7a/8a, 7a/8b, 7b/8a, or 7b/8b that encode isoforms SULT1C3a-d, respectively (Freimuth et al., 2004). The SULT1C3 mRNA reference sequence (NM_001008743) contains exons 7b/8b, which encodes SULT1C3d. This transcript was not detected in any of 20 human tissues, including liver and kidney, that were used for expression profiling (Freimuth et al., 2004; Meinl et al., 2008a). Using reverse transcription-polymerase chain reaction and rapid amplification of cDNA ends (RACE) analysis, we detected SULT1C3 mRNA containing exons 7a/8a, encoding SULT1C3a, in human small intestine and colon and in LS180 colorectal adenocarcinoma cells (Duniec-Dmuchowski et al., 2014). We also reported that SULT1C3 expression in LS180 cells is regulated by activators of several transcription factors, including peroxisome proliferator-activated receptors (PPARs), farnesoid X receptor (FXR), liver X receptor (LXR), pregnane X receptor (PXR), and aryl hydrocarbon receptor (AhR) (Rondini et al., 2014).

There are three PPARs: PPARα, PPARδ, and PPARγ. These nuclear receptors are differentially expressed in mammalian tissues, including small and large intestine, where they regulate a variety of genes that are involved in energy metabolism, lipid metabolism, and other cellular processes (Huin et al., 2000; Su et al., 2007). Lipid- and xenobiotic-sensing receptors, including PPARs, are known to regulate expression of human SULTs (Fang et al., 2005, 2007; Jiang et al., 2005; Echchgadda et al., 2007; Kodama et al., 2011). For example, we previously reported that PPARα regulates human SULT2A1 transcription through a peroxisome proliferator response element (PPRE) in the distal 5′-flanking region of the SULT2A1 gene (Fang et al., 2005). In this study, we explored the regulation of SULT1C3 by PPAR activation in LS180 cells. Specifically, we established regulation by PPARγ and identified a functional PPRE in a polymorphic region of the SULT1C3 promoter.

Materials and Methods

Materials. Ciprofibrate was provided by Sterling Winthrop Pharmaceuticals Research Division (Rennselaer, NY). 4-[[[2-[3-fluoro-4-(trifluoromethyl)phenyl]-4-methyl-5-thiazolyl]methyl]-thio]-2-methylphenoxyacetic acid (GW0742), 3-[3-[[[2-chloro-3-(trifluoromethyl)phenyl]methyl](2,2-diphenylethyl)amino]propoxy]-benzeneacetic acid hydrochloride (GW3965), 3-[2-[2-chloro-4-[[3-(2,6-dichlorophenyl)-5-(1-methylethyl)-4-isoxazolyl]methoxy]phenyl]-ethenyl]benzoic acid (GW4064), and 2-[[4-[2-[[(cyclohexylamino)-carbonyl](4-cyclohexylbutyl)amino]ethyl]phenyl]thio]-2-methylpropanoic acid (GW7647) were purchased from Tocris Biosciences (Minneapolis, MN). Rosiglitazone, rifampicin, and dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich (St. Louis, MO), and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) was purchased from Midwest Research Institute (Kansas City, MO). Cell culture media and supplements and LipofectAMINE 2000 were purchased from Life Technologies (Grand Island, NY) Oligonucleotides were purchased from Integrated DNA Technologies (IDT, Coralville, IA).

Cell Culture. LS180 cells were purchased from the American Type Culture Collection (Manassas, VA) and cultured in Eagle's minimum essential medium (MEM) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 1 mM sodium pyruvate, MEM nonessential amino acids, 100 U/ml penicillin, and 100 μg/ml streptomycin. HEK293A cells were purchased from Life Technologies and cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100μg/ml streptomycin. Cells were maintained in a humidified atmosphere of 5% CO_2 and 95% air at 37°C.

Preparation of SULT1C3 Reporter Plasmids. Genomic DNA was isolated from MCF10A and LS180 cells using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA). Genomic DNA (100 ng), Herculase II Fusion DNA polymerase (Agilent Technologies, Santa Clara, CA), and a primer set that was predicted to amplify a 2.8-kb fragment of the SULT1C3 5′-flanking region (nt −2780: +38), as identified by our previous 5′-RACE analysis, were used for polymerase chain reaction (PCR) (Duniec-Dmuchowski et al., 2014). The resulting PCR fragments were ligated into the KpnI and XhoI sites of the promoterless pGL4.10[luc2] firefly luciferase reporter plasmid (Promega Corporation, Madison, WI). The plasmid containing the 2.8-kb insert was used as template to prepare a construct containing ~1 kb of the SULT1C3 5′-flanking sequence (nucleotides −1008: +38), which was subsequently used as template to prepare a series of deletion constructs that were designed based on the positions of three PPRE motifs, at nucleotides −769, −446, and −383, that were predicted by computational analysis using MatInspector (Genomatix, Ann Arbor, MI) (Quandt et al., 1995; Cartharius et al., 2005). All primer sequences are shown in Table 1. The sequences of all SULT1C3 clones were confirmed using the services of the Applied Genomics Technology Center at Wayne State University.

Site-Directed Mutagenesis of PPRE Motifs. Mutations were introduced into the three predicted PPREs using the wild-type or singly mutated (at the −446 PPRE) SULT1C3 1-kb construct as template and the QuikChange II Site-Directed Mutagenesis Kit according to the manufacturer's instructions (Agilent Technologies, Santa Clara, CA). The mutagenic primers are listed in Table 1.

Transient Transfection Analysis and Treatments. Approximately 250,000 LS180 cells/well in 1 ml of supplemented MEM were plated into 12-well plates. Cells were transfected 48–72 hours after seeding with a complex containing 4 μl of LipofectAMINE 2000, 1.6 μg of a firefly luciferase reporter plasmid, and 1ng pRL-CMV (Promega) per well diluted in 400 μl of Opti-MEM (Life Technologies). Twenty-four hours after transfection, fresh supplemented MEM was added containing either DMSO (0.1% final concentration) or a transcription factor activator (at concentrations indicated in the following text and figure legends). Treatment medium was changed after 24 hours. Cells were lysed and collected after 48 hours of treatment, and firefly and *Renilla* luciferase activities were measured using the Dual-Luciferase Reporter Assay System (Promega) and a Glomax Luminometer (Promega). For each sample, the firefly luciferase value was normalized to the corresponding *Renilla* luciferase value.

PPARγ in Vitro Binding Assay. A PPARγ expression plasmid (pTR151) was provided by Dr. Todd Leff (Wayne State University). HEK293A cells were plated into 100-mm dishes and transiently transfected with a complex containing 50 μl of LipofectAMINE 2000, 4 μg of PPARγ expression plasmid, 0.8 μg of a Tet-off plasmid, and 15.2μg of pBluescript II KS^+ (Agilent Technologies). Forty-eight hours after transfection, cells were harvested and nuclear extracts were prepared using the NucBuster Protein Extraction Kit (Millipore, Billerica, MA). Competitive binding experiments were performed using the TransAM PPARγ Kit (Active Motif, Carlsbad, CA), an enzyme-linked immunosorbent assay. Each well in the 96-well plate contained an immobilized biotinylated oligonucleotide probe that included a consensus PPRE sequence. Competitor oligonucleotides containing the wild-type or mutated SULT1C3 PPRE (at nt −769) or CYP4A1 PPRE as positive control (Aldridge et al., 1995) were designed (sequences are shown in Table 1), purchased, and annealed by heating at 95°C for 5 minutes, followed by slowly cooling to room temperature. Binding assays were performed according to the manufacturer's instructions. Incubations included 5 μg of nuclear protein extract and 0, 30, or 100 pmol of competitor oligonucleotide and were performed for 1 hour. PPARγ binding to the biotinylated probe was determined by colorimetric analysis using a microplate reader (CLARIOstar, BMG LABTECH, Cary, NC) at wavelengths of 450 and 655 nm.

RNA Interference. LS180 cells were plated into 12-well plates and cotransfected with 1.6 μg of the SULT1C3 1-kb reporter plasmid

TABLE 1
Primers used to prepare reporter constructs and competitor oligonucleotides used for PPARγ in vitro binding assay

Name	Sequence
Primers for reporters	
SULT1C3-TSS reverse[a]	5′-GGG CTC GAG GCT CCA GGA CAC TGT GCA AGC AA-3′
SULT1C3-2.8Kb forward	5′-GGG GGT ACC TCT GGT CCT CCT TCA TTC CCG CAA-3′
SULT1C3-1Kb forward	5′-GGG GGT ACC ATG CTC TAC ATA ATT CAC GTC-3′
SULT1C3-DEL1 forward	5′-GGG GGT ACC ACA GAG GAC AGA CAA TGT AAA T-3′
SULT1C3-DEL2 forward	5′-GGG GGT ACC TTT TAT TAC AGG CCT TGT GGT-3′
SULT1C3-DEL3 forward	5′-GGG GGT ACC TTT CTA CAG GGT CAA AGG GA-3′
SULT1C3-DEL4 forward	5′-GGG GGT ACC AAC AGG ATG AAA TAA TTG TGC-3′
SULT1C3-MUT1 sense[b]	5′-GGA GTT AAG TAA ATA TTG TAC AGA AGG TAT TGT TAA AAT TCC ATA TAT TTA CAT TGTT CTG TCC TCT GTT TTG CAA-3′
SULT1C3-MUT2 sense	5′-CCG TAG TTA AAA TTG GTG TAG AAG AAA AAG CTT TTT AGG AAA CCA CAA GGC CTGT TAA AAC-3′
SULT1C3-MUT3 sense	5′-ACT TGC ACA ATT ATT TCA TCC TGT TCC CTG GAT CCC TGT AGA AAA TAT ATT CTA TTG CCT CT-3′
Competitor oligonucleotides for PPARγ in vitro binding assay	
SULT1C3-WT PPRE sense[b]	5′- AAC AAT GAA CTC TGT ACA ATA TTT -3′
SULT1C3-MUT PPRE sense	5′- AAC AAT ACC TTC TGT ACA ATA TTT -3′
CYP4A1-WT PPRE sense	5′- GAA ACT AGG GTA AAG TTC AGT GAG -3′
CYP4A1-MUT PPRE sense	5′- GAA ACT CGG AGC ACG TTA AGT GAG -3′

[a]The same reverse primer was used to prepare the 2.8-kb, 1.9-kb, 1-kb, DEL1, DEL2, DEL3, and DEL4 SULT1C3 fragments.
[b]Only sense-strand sequences are shown for site-directed mutagenesis primers and in vitro binding assay oligonucleotides.

and 20 pmol of a small interfering RNA (siRNA) pool targeting PPARα, PPARδ, or PPARγ mRNA (ON-TARGETplus SMARTpool siRNA; Dharmacon, Lafayette, CO) or a negative control siRNA pool (ON-TARGETplus nontargeting control siRNA, Dharmacon). Cells were transfected using 4 μl of LipofectAMINE 2000 and 1 ng pRL-CMV per well. Twenty-four hours after transfection, cells were treated with DMSO (final concentration of 0.1%), rosiglitazone (1 μM), GW7647 (10 μM), or GW0742 (10 μM). Treatment medium was changed after 24 hours. After 48-hour treatment, cells were collected to measure firefly and *Renilla* luciferase activities as described above. A luciferase reporter containing the PPRE from the promoter of the CYP4A1 gene was used as a positive control for detection of PPAR knockdown (Kocarek and Mercer-Haines, 2002).

Statistical Analysis. Statistical analysis was performed using GraphPad Prism (version 6; GraphPad, La Jolla, CA). Data were analyzed using one-way analysis of variance followed by the Neuman-Keuls post-hoc test. $P < 0.05$ was considered significantly different. Data are presented as means ± S.D. relative to DMSO control. In each experiment, all treatments were performed in triplicate. Each experiment was repeated three times.

Results

Evaluation of the SULT1C3 5′-Flanking Region for Responsiveness to Transcription Factor Activators. We previously reported that SULT1C3 mRNA levels in LS180 cells were increased by treatments with several transcription factor activators, including ciprofibrate (PPARα), rosiglitazone (PPARγ), GW4064 (FXR), GW3965 (LXR), rifampicin (PXR), and TCDD (AhR) (Rondini et al., 2014). To determine the mechanisms underlying the regulation of SULT1C3 by these transcription factor activators, luciferase reporter constructs containing portions of the 5′-flanking region of SULT1C3 were prepared. While attempting to amplify a 2.8-kb fragment (−2789: +36) using human genomic DNA from two different sources, LS180 cells and the MCF10A mammary epithelial cell line, both the expected 2.8-kb fragment and a 1.9-kb fragment were generated from LS180 cells, whereas only the 1.9-kb fragment was amplified from MCF10A cells (Fig. 1A). Sequencing of the two fragments revealed that the 1.9-kb fragment had an internal deletion of 863 nt (−1008: −146) relative to the 2.8-kb fragment (Fig. 1A). LS180 cells were transiently transfected with a reporter plasmid containing the 2.8-kb or 1.9-kb fragment and then treated for 48 hours with 100 μM ciprofibrate, 10μM rosiglitazone, 1 μM GW4064, 10 μM GW3965, 30 μM rifampicin, or 0.01 μM TCDD. Of these treatments, ciprofibrate and rosiglitazone significantly increased luciferase activity from the 2.8-kb reporter construct by ~1.5-fold and 3.0-fold, respectively, but did not increase activity from the 1.9-kb reporter, suggesting that the 863-nt deletion region contained essential elements for PPAR-mediated activation of SULT1C3 transcription (Fig. 1B). In support of this conclusion, treatment with rosiglitazone, the strongest activator of SULT1C3 transcription, also significantly increased luciferase activity from a reporter containing only the 863-nt deletion and more proximal promoter region (1-kb construct; nt −1008: +36) (Fig. 2A). Whereas GW3965 and GW4064 treatments significantly increased luciferase activity from both the 2.8-kb and 1.9-kb reporter constructs, these effects could not be attributed to SULT1C3 transactivation since these treatments also significantly activated the empty reporter plasmid (Fig. 1B). Rifampicin and TCDD treatments did not increase expression from any of the reporter constructs (Fig. 1B). Therefore, transcriptional regulation of SULT1C3 by AhR, FXR, LXR, or PXR was not considered further in this study.

Concentration-Dependent Effects of PPARα, PPARδ, and PPARγ Agonists on SULT1C3 Transcriptional Activation. To evaluate the SULT1C3 863-nt deletion region further for its responsiveness to PPAR activation, we determined the concentration-dependent effects of agonists for the three PPARs on SULT1C3 reporter activity. For these studies, LS180 cells were transfected with the 1-kb reporter construct and then treated with varying concentrations (0.1–10 μM) of rosiglitazone (PPARγ), GW7647 (PPARα), or GW0742 (PPARδ). Rosiglitazone treatment maximally increased reporter activity at the lowest concentration tested, which is consistent with its high potency for PPARγ (Fig. 2B). Although GW7647 and GW0742 also increased reporter activity, the submicromolar concentrations that would reflect the high reported potencies of these compounds for their respective receptors had little or no effect, whereas the higher concentrations that might reflect cross-activation of another

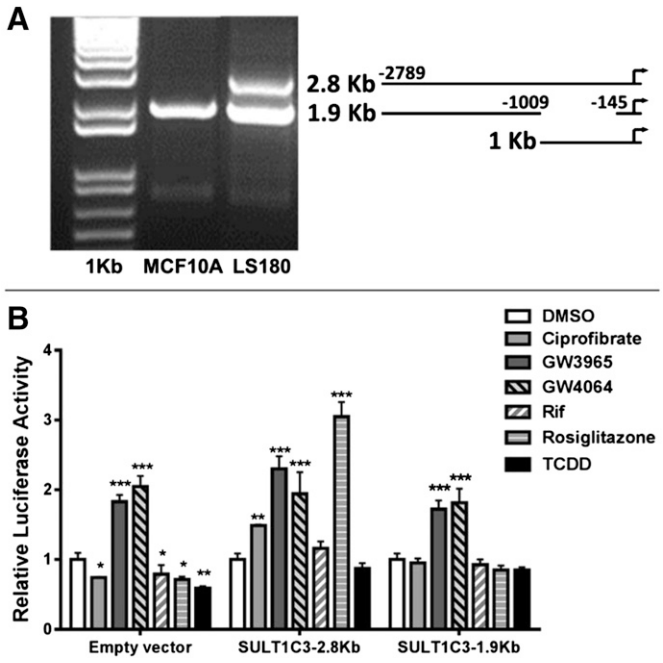

Fig. 1. Impact of a deletion in the 5′-flanking region of the SULT1C3 gene on its regulation by transcription factor activators. (A) PCR was performed with primers designed to amplify a 2.8-kb fragment of the SULT1C3 5′-flanking region using genomic DNA from MCF10A or LS180 cells. The PCR products were resolved on a 1% agarose gel (1-kb ladder also shown). A schematic representation of the 2.8-, 1.9-, and 1-kb SULT1C3 5′-flanking fragments is shown adjacent to the gel image. (B) LS180 cells were transiently transfected with the SULT1C3-2.8-kb or 1.9-kb luciferase reporter plasmid or with the pGL4.10 [luc2] empty reporter vector and then treated with DMSO (0.1%), ciprofibrate (PPARα agonist, 100 μM), GW3965 (LXR, 10 μM), GW4064 (FXR, 1 μM), rifampicin (PXR, 30 μM), rosiglitazone (PPARγ, 10 μM), or TCDD (AhR, 0.01 μM) for 48 hours. The cells were then harvested for measurement of luciferase activities. Each column represents the mean ± S.D. of normalized (Firefly/*Renilla*) luciferase measurements relative to DMSO control (n = 3 wells per treatment) from one cell culture experiment. Similar data were obtained in two additional independent experiments. *, **, ***Significantly different from DMSO-treated controls at $P < 0.05$, 0.01, and 0.001, respectively.

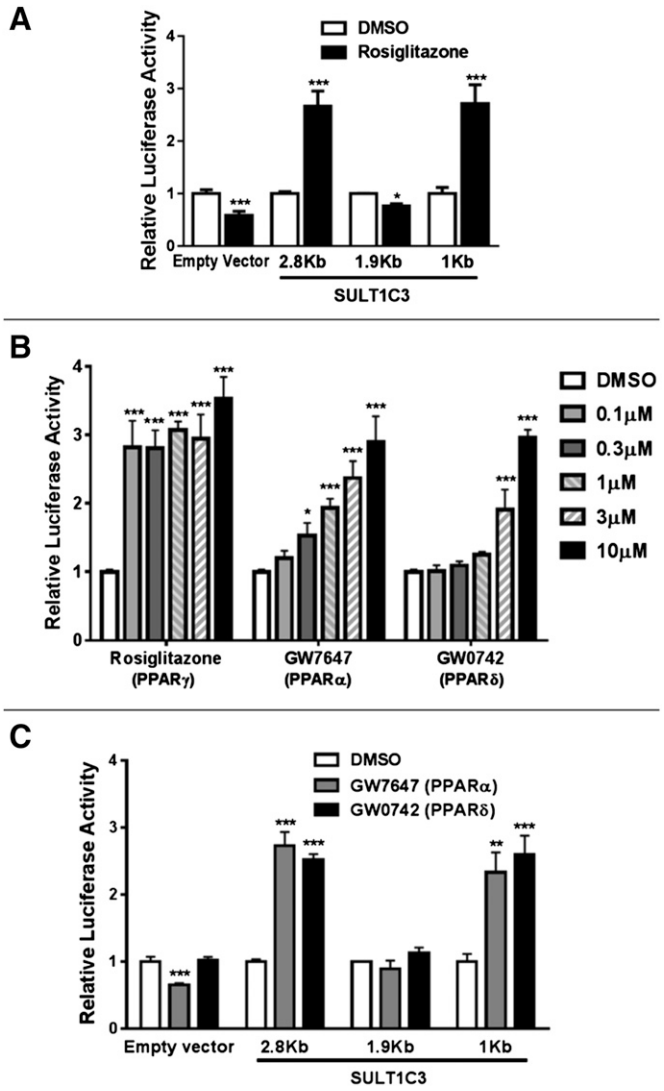

Fig. 2. Effects of PPAR agonists on transcription of SULT1C3 reporter constructs. (A) LS180 cells were transiently transfected with a luciferase reporter plasmid containing either the 2.8-kb or 1.9-kb SULT1C3 5′-flanking region fragment, with the 1-kb reporter containing the deleted region or with control empty vector. Twenty-four hours after transfection, the cells were treated with DMSO (0.1%) or rosiglitazone (10 μM) for 48 hours. (B) LS180 cells transfected with the 1-kb reporter were treated with DMSO (0.1%) or with 0.1–10 μM rosiglitazone (PPARγ agonist), GW7647 (PPARα), or GW0742 (PPARδ) for 48 hours. (C) LS180 cells transfected with the 2.8-kb, 1.9-kb, or 1-kb reporter or with control empty vector were treated with DMSO (0.1%), GW7647 (10μM), or GW0742 (10μM). The cells were then harvested for measurement of luciferase activities. Each column represents the mean ± S.D. of normalized (Firefly/*Renilla*) luciferase measurements relative to the corresponding DMSO control (n = 3 wells per treatment) from one cell culture experiment. Similar data were obtained in two additional independent experiments. *, **, ***Significantly different from DMSO-treated control at $P <$ 0.05, 0.01, and 0.001, respectively.

PPAR produced concentration-dependent increases that reached ~3-fold at 10 μM, which is comparable to the magnitude of increase produced by rosiglitazone (Fig. 2B). Treatment with 10 μM GW7647 and GW0742 also induced luciferase activity from the reporter containing the longer 2.8-kb region but not the 1.9-kb construct lacking the 863-nt fragment (Fig. 2C), as seen for ciprofibrate and rosiglitazone (Fig. 1B).

Identification of a Functional PPRE within the 863nt Deletion Region of the SULT1C3 Gene. Computational analysis identified three putative PPREs within the 1Kb reporter fragment (−1008: +36) located −769, −446, and −383 nt upstream from the transcription start site. To determine the functionality of these predicted PPREs, we first prepared four reporter constructs that progressively deleted the three PPREs from the 1-kb fragment and transfected them into LS180 cells. Treatment with rosiglitazone (1 μM), GW7647 (10 μM), or GW0742 (10 μM) for 48 hours increased the luciferase activity of the 1-Kb construct and deletion construct containing all three PPREs (−808: +36) by 3- to 5- fold relative to DMSO-treated controls but did not increase the activity of the three reporters that lacked the first PPRE at −769 (Fig. 3A). These data implicate the PPRE at nt −769 as an essential element for obtaining PPAR-mediated SULT1C3 transactivation.

To confirm the importance of the PPRE at −769 and determine the involvement of the other two predicted PPREs in the transcriptional activation of SULT1C3, mutations were introduced into each of the PPREs. LS180 cells were transfected with reporters containing either the wild-type 1-kb fragment or the 1-kb fragment with one or two mutated PPREs. Mutation of the distal PPRE (at nt −769) eliminated the response of the 1-kb reporter to rosiglitazone, GW7647, and GW0742 treatments (Fig. 3B); however, the PPAR

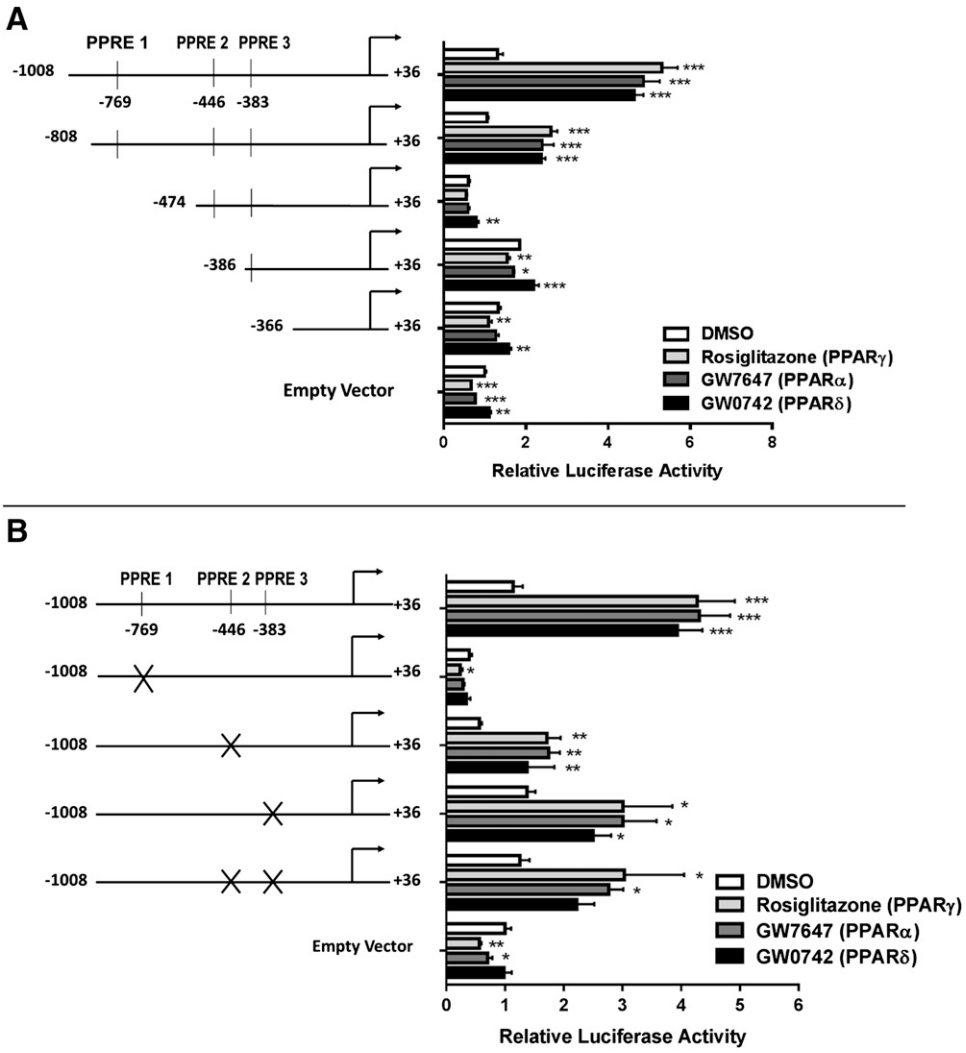

Fig. 3. Evaluation of three computationally predicted PPREs within the deleted region of the SULT1C3 5′-flanking region. LS180 cells were transiently transfected with a series of reporter plasmids with progressive 5′-deletions from the 1-kb SULT1C3 reporter plasmid that were designed based on the positions of computationally predicted PPREs (A) or with the 1-kb SULT1C3 reporter plasmid containing either the wild-type sequence or site-directed mutations at one or two of the predicted PPREs (B). Transfected cells were treated with DMSO (0.1%), rosiglitazone (1 μM), GW7647 (10 μM), or GW0742 (10 μM) for 48 hours and then harvested for measurement of luciferase activities. Each column represents the mean ± S.D. of normalized (Firefly/*Renilla*) luciferase measurements relative to the DMSO-treated, empty vector-transfected group (n = 3 wells per treatment) from one cell culture experiment. Similar data were obtained in two additional independent experiments. *, **, ***Significantly different from DMSO-treated controls at $P < 0.05$, 0.01, and 0.001, respectively.

agonists were all able to produce significant activation of reporters in which one or both of the more proximal PPREs (at nt −446 and −383) were mutated (Fig. 3B). Mutation of the PPRE at −446 did cause some reduction in reporter activity; however, since both the control (i.e., DMSO-treated) and PPAR agonist-mediated activities were reduced, the PPAR agonist-mediated fold increases were comparable to those seen for the wild-type reporter. These data indicate that only the PPRE at −769 is essential for obtaining PPAR-mediated activation of SULT1C3 transcription.

A competitive enzyme-linked immunosorbent-based in vitro DNA-binding assay was used to determine the ability of PPARγ to bind directly to the distal PPRE. As shown in Fig. 4, the addition of 30 or 100 pmol of a double-stranded competitor oligonucleotide containing the wild-type SULT1C3 PPRE, but not the mutated SULT1C3 PPRE, significantly decreased the amount of PPARγ that bound to a biotinylated capture probe. This level of inhibition was approximately the same as that produced by a competitor containing the CYP4A1 PPRE.

Predominance of PPARγ in the Transcriptional Regulation of SULT1C3. Rosiglitazone, GW7647, and GW0742 are potent and selective agonists of PPARγ, PPARα, and PPARδ, respectively, although each agonist can cross-activate nontarget PPAR receptors at sufficiently high concentrations. As shown in Fig. 2, whereas rosiglitazone increased SULT1C3 reporter expression at low concentrations consistent with PPARγ activation, the effects of GW7647 and GW0742 were evident only at relatively high concentrations, suggesting that these compounds might have increased SULT1C3 transcription by cross-activating PPARγ rather than by activating their target receptors. To test this possibility, we assessed the impact of siRNA-mediated knockdown of each PPAR on activation of the 1-kb reporter construct by 1 μM rosiglitazone, 10 μM GW7647, and 10 μM GW0742. The knockdown of PPARγ decreased activation of the positive control CYP4A1-PPRE reporter and the SULT1C3 1-kb reporter by almost 90%, not only by rosiglitazone, but also by GW7647 and GW0742 (Fig. 5A); however, knockdown of PPARα or PPARδ had little to no effect on reporter activation by rosiglitazone, GW7647, or GW0742 (Fig. 5, B and C). These data indicate that the effects of all three PPAR agonists on SULT1C3 transcription can mainly be attributed to the activation of PPARγ.

Discussion

The intestine is a major portal of entry for many drugs, environmental chemicals, and other xenobiotics, and the

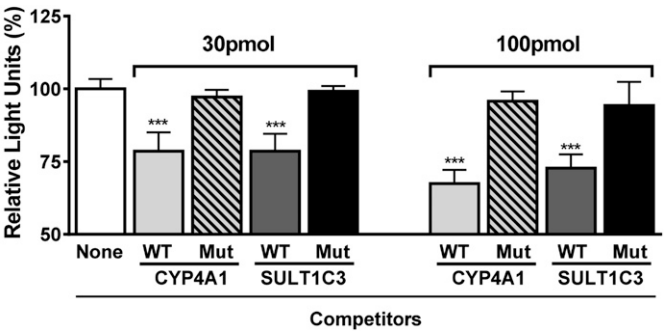

Fig. 4. In vitro binding of PPARγ to the predicted SULT1C3 PPRE at nt −769. In vitro binding was determined by incubating a biotinylated oligonucleotide containing a consensus PPRE with PPARγ-containing nuclear extract in the absence or presence of unbiotinylated competitor oligonucleotide (30 or 100 PM) containing wild-type or mutated CYP4A1 or SULT1C3 PPRE, as described in *Methods*. Each column represents the mean ± S.D. absorbance relative to the control absorbance (no competitor added; $n = 4$). ***Significantly different from control at $P < 0.001$.

intestine is therefore equipped with many xenobiotic-metabolizing enzymes, including several SULTs. SULT1A1, SULT1A3, and SULT1B1 are abundantly expressed throughout the gastrointestinal tract, whereas SULT1E1 and SULT2A1 are expressed in the jejunum, ileum, and cecum (Teubner et al., 2007; Riches et al., 2009). SULTs expressed in the intestine, including SULT1A1 and SULT2A1, are involved in endogenous molecule metabolism, xenobiotic elimination, and procarcinogen activation. SULT1A1 can metabolize hormones such as di-iodothyronine and estrogens (Falany, 1997; Richard et al., 2001), drugs such as minoxidil (Falany and Kerl, 1990) and troglitazone (Honma et al., 2002), and procarcinogens such as the heterocyclic amine 2-amino-3-methyl-9H-pyrido [2,3-b]indole (Glatt et al., 2004), whereas SULT2A1 can sulfonate bile acids, dehydroepiandrosterone, and benzylic alcohol procarcinogens (Falany et al., 1995; Glatt et al., 1995). SULT1C enzymes are also expressed in the gastrointestinal tract. SULT1C2 is expressed in stomach, duodenum, and colon, whereas SULT1C4 has been detected in some human intestinal cell lines (Dooley et al., 2000; Meinl et al., 2008b), although not in LS180 cells (Rondini et al., 2014).

Studies to evaluate SULT1C3 (isoform d) activity have indicated that this enzyme can sulfonate large benzylic alcohols, including metabolites of polycyclic aromatic hydrocarbons, and the steroid-related compounds α-zearalenol and lithocholic acid (Allali-Hassani et al., 2007; Meinl et al., 2008a). Also, a recent study reported that SULT1C3 had the lowest K_m and highest V_{max} of 12 human SULTs tested for sulfonation of tolvaptan, which is a selective vasopressin V_2-receptor antagonist that possesses a benzylic hydroxy group (Fang et al., 2015).

We previously reported that SULT1C3 expression in LS180 cells is increased in response to treatment with activators of AhR, FXR, LXR, PPARα, PPARγ, and PXR (Rondini et al., 2014); however, we report here that only PPAR agonists activated SULT1C3 transcription through sequence information contained within a 2.8-kb fragment (−2789: +36) of the gene's 5′-flanking region. This finding implies that the *cis*-elements controlling SULT1C3 expression by the other transcription factors are located in other regions of the SULT1C3 gene, possibly further upstream or within the

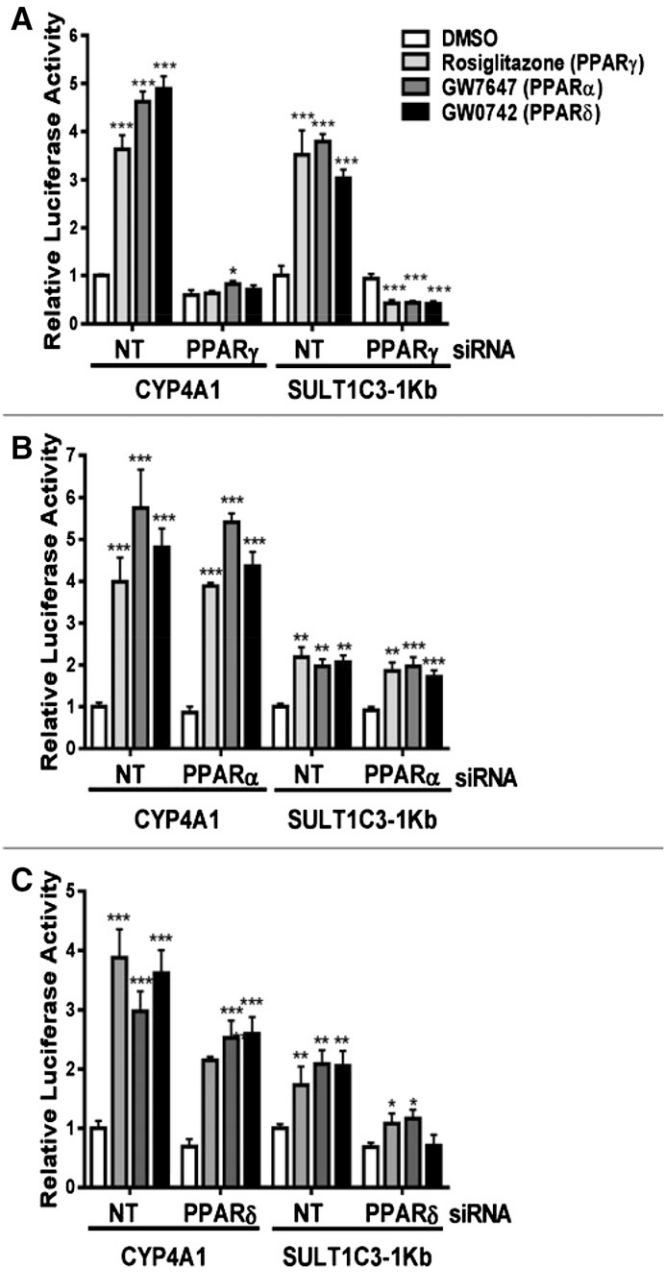

Fig. 5. Effect of PPAR knockdowns on SULT1C3 transcriptional activation by different classes of PPAR agonist. LS180 cells were transiently cotransfected with either the SULT1C3 1-kb or the CYP4A1 PPRE reporter plasmid (positive control) and 20 pmol of either nontargeting (NT) siRNA or siRNA targeting PPARγ (A), PPARα (B), or PPARδ (C). Twenty-four hours after transfection cells were treated with DMSO (0.1%), rosiglitazone (1 μM), GW7647 (10 μM), or GW0742 (10 μM) for 48 hours and then harvested for measurement of luciferase activities. Each column represents the mean ± S.D. of normalized (Firefly/*Renilla*) luciferase measurements (three wells per treatment). Similar data were obtained in two additional independent experiments. *, **, ***Significantly different from DMSO-treated controls at $P < 0.05$, 0.01, and 0.001, respectively.

7.1-kb intron that separates noncoding exon 1 from exon 2 (Duniec-Dmuchowski et al., 2014). Computational analysis of the SULT1C3 gene using MatInspector indicated the presence of several candidate binding sites for AhR, FXR, LXR, and PXR in the region spanning from 10-kb upstream of the transcription start site through intron 1.

While attempting to amplify a 2.8-kb fragment of the SULT1C3 5′-flanking region, we found that amplicons of two different sizes were generated from LS180 genomic DNA; the expected 2.8-kb fragment and a smaller fragment with an internal 863-nt deletion, from −1008 to −146 relative to the transcription start site; however, only the smaller fragment was detected in MCF10A cells, indicating that there was variability in the SULT1C3 5′-flanking sequence among sources of genomic DNA. This sequence variability appears to be attributable to a deletion polymorphism since the 1000 Genomes Project includes this structural variant in its database (esv3591922) (Abecasis et al., 2012). Since this deletion region contains a functional PPARγ binding site, it is plausible that SULT1C3 expression may vary among individuals, depending on whether they carry the variant allele.

The three PPARs are expressed in fetal and adult human intestine (Abbott et al., 2010), and we have shown that PPARα and PPARγ are expressed in LS180 cells (PPARδ was not evaluated) (Rondini et al., 2014). In the current investigation, we found that rosiglitazone, a potent and selective PPARγ agonist, significantly increased the activities of luciferase reporter plasmids containing the deletion region of the SULT1C3 5′-flanking region (i.e., the 2.8-kb and 1-kb reporters). Although the luciferase activity of these reporters was also increased by PPARα and PPARδ agonist treatments, these effects were probably attributable to cross-activation of PPARγ since: 1) relatively high concentrations of the PPARα and PPARδ agonists were needed to induce the reporter activity, and 2) siRNA targeting PPARγ abolished reporter activation by all PPAR agonists while siRNA targeting PPARα or PPARδ had little effect. These data indicate that PPARγ is the predominant PPAR that regulates SULT1C3 transcription in LS180 cells.

PPARγ is highly expressed in the various regions of human intestine, at levels that are comparable to those detected in adipocytes, and this transcription factor could play a role in gastrointestinal morphogenesis during fetal development (Fajas et al., 1997; Huin et al., 2000; Abbott et al., 2010). In the intestine, PPARγ signaling has been linked to growth arrest, apoptosis, and differentiation (Sarraf et al., 1998; Gupta et al., 2001, 2003; Drori et al., 2005). Several studies classified PPARγ-regulated genes into three functional categories: regulation of lipid metabolism, signal transduction, and motility and adhesion (Chen et al., 2006; Bush et al., 2007; Su et al., 2007). SULT1C3 could play a role in intestinal physiology by metabolizing one or more endogenous molecules that function in the regulation of these PPARγ-regulated processes.

In summary, we have identified a functional PPRE in the 5′-flanking region of the SULT1C3 gene, thereby establishing SULT1C3 as a direct PPARγ target in intestinal cells. This finding implies that SULT1C3 could play a role in PPARγ-regulated processes associated with intestinal development and function. Since the PPRE is located within a polymorphic region of the SULT1C3 gene, our findings also provide a mechanistic rationale to hypothesize that there could be considerable differences among individuals in the intestinal expression of SULT1C3. Further studies are needed to establish the genotype-phenotype relationship between the presence of the PPRE and intestinal SULT1C3 expression. The structural variant lacking the PPRE appears to be a fairly common polymorphism (the overall allele frequency of esv3591922 in the 1000 Genomes Database is reported to be 0.3329), with variable frequency in different populations (allele frequencies for African, Ad Mixed American, East Asian, European, and South Asian superpopulations of 0.2852, 0.3329, 0.1091, 0.4394, and 0.5184, respectively). It is possible that interindividual differences in intestinal SULT1C3 expression could have pharmacologic and toxicologic implications, for example, by modifying the risk for intestinal bioactivation of procarcinogenic molecules.

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Authorship Contributions

Participated in research design: Dubaisi, Kocarek, Runge-Morris.
Conducted experiments: Dubaisi, Fang.
Performed data analysis: Dubaisi, Kocarek.
Wrote or contributed to the writing of the manuscript: Dubaisi, Kocarek, Runge-Morris.

References

Abbott BD, Wood CR, Watkins AM, Das KP, and Lau CS (2010) Peroxisome proliferator-activated receptors alpha, beta, and gamma mRNA and protein expression in human fetal tissues. *PPAR Res* **2010**:2010.
Abecasis GR, Auton A, Brooks LD, DePristo MA, Durbin RM, Handsaker RE, Kang HM, Marth GT, and McVean GA; 1000 Genomes Project Consortium (2012) An integrated map of genetic variation from 1,092 human genomes. *Nature* **491**:56–65.
Allali-Hassani A, Pan PW, Dombrovski L, Najmanovich R, Tempel W, Dong A, Loppnau P, Martin F, Thornton J, Edwards AM, et al. (2007) Structural and chemical profiling of the human cytosolic sulfotransferases. *PLoS Biol* **5**:e97.
Aldridge TC, Tugwood JD, and Green S (1995) Identification and characterization of DNA elements implicated in the regulation of CYP4A1 transcription. *Biochem J* **306**:473–479.
Blanchard RL, Freimuth RR, Buck J, Weinshilboum RM, and Coughtrie MW (2004) A proposed nomenclature system for the cytosolic sulfotransferase (SULT) superfamily. *Pharmacogenetics* **14**:199–211.
Bush CR, Havens JM, Necela BM, Su W, Chen L, Yanagisawa M, Anastasiadis PZ, Guerra R, Luxon BA, and Thompson EA (2007) Functional genomic analysis reveals cross-talk between peroxisome proliferator-activated receptor gamma and calcium signaling in human colorectal cancer cells. *J Biol Chem* **282**:23387–23401.
Cartharius K, Frech K, Grote K, Klocke B, Haltmeier M, Klingenhoff A, Frisch M, Bayerlein M, and Werner T (2005) MatInspector and beyond: promoter analysis based on transcription factor binding sites. *Bioinformatics* **21**:2933–2942.
Chen L, Bush CR, Necela BM, Su W, Yanagisawa M, Anastasiadis PZ, Fields AP, and Thompson EA (2006) RS5444, a novel PPARgamma agonist, regulates aspects of the differentiated phenotype in nontransformed intestinal epithelial cells. *Mol Cell Endocrinol* **251**:17–32.
Dooley TP, Haldeman-Cahill R, Joiner J, and Wilborn TW (2000) Expression profiling of human sulfotransferase and sulfatase gene superfamilies in epithelial tissues and cultured cells. *Biochem Biophys Res Commun* **277**:236–245.
Drori S, Girnun GD, Tou L, Szwaya JD, Mueller E, Xia K, Shivdasani RA, and Spiegelman BM (2005) Hic-5 regulates an epithelial program mediated by PPARgamma. *Genes Dev* **19**:362–375.
Duniec-Dmuchowski Z, Rondini EA, Tibbs ZE, Falany CN, Runge-Morris M, and Kocarek TA (2014) Expression of the orphan cytosolic sulfotransferase SULT1C3 in human intestine: characterization of the transcript variant and implications for function. *Drug Metab Dispos* **42**:352–360.
Echchgadda I, Song CS, Oh T, Ahmed M, De La Cruz IJ, and Chatterjee B (2007) The xenobiotic-sensing nuclear receptors pregnane X receptor, constitutive androstane receptor, and orphan nuclear receptor hepatocyte nuclear factor 4alpha in the regulation of human steroid-/bile acid-sulfotransferase. *Mol Endocrinol* **21**: 2099–2111.
Fajas L, Auboeuf D, Raspé E, Schoonjans K, Lefebvre AM, Saladin R, Najib J, Laville M, Fruchart JC, Deeb S, et al. (1997) The organization, promoter analysis, and expression of the human PPARgamma gene. *J Biol Chem* **272**:18779–18789.
Falany CN (1997) Enzymology of human cytosolic sulfotransferases. *FASEB J* **11**: 206–216.
Falany CN, Comer KA, Dooley TP, and Glatt H (1995) Human dehydroepiandrosterone sulfotransferase. Purification, molecular cloning, and characterization. *Ann N Y Acad Sci* **774**:59–72.
Falany CN and Kerl EA (1990) Sulfation of minoxidil by human liver phenol sulfotransferase. *Biochem Pharmacol* **40**:1027–1032.
Fang HL, Strom SC, Cai H, Falany CN, Kocarek TA, and Runge-Morris M (2005) Regulation of human hepatic hydroxysteroid sulfotransferase gene expression by the peroxisome proliferator-activated receptor alpha transcription factor. *Mol Pharmacol* **67**:1257–1267.

Fang HL, Strom SC, Ellis E, Duanmu Z, Fu J, Duniec-Dmuchowski Z, Falany CN, Falany JL, Kocarek TA, and Runge-Morris M (2007) Positive and negative regulation of human hepatic hydroxysteroid sulfotransferase (SULT2A1) gene transcription by rifampicin: roles of hepatocyte nuclear factor 4alpha and pregnane X receptor. *J Pharmacol Exp Ther* **323**:586–598.

Fang JL, Wu Y, da Costa GG, Chen S, Chitranshi P, and Beland FA (2015) Human sulfotransferases enhance the cytotoxicity of tolvaptan. *Toxicol Sci* **150**:27–39.

Freimuth RR, Raftogianis RB, Wood TC, Moon E, Kim UJ, Xu J, Siciliano MJ, and Weinshilboum RM (2000) Human sulfotransferases SULT1C1 and SULT1C2: cDNA characterization, gene cloning, and chromosomal localization. *Genomics* **65**: 157–165.

Freimuth RR, Wiepert M, Chute CG, Wieben ED, and Weinshilboum RM (2004) Human cytosolic sulfotransferase database mining: identification of seven novel genes and pseudogenes. *Pharmacogenomics J* **4**:54–65.

Glatt H, Boeing H, Engelke CE, Ma L, Kuhlow A, Pabel U, Pomplun D, Teubner W, and Meinl W (2001) Human cytosolic sulphotransferases: genetics, characteristics, toxicological aspects. *Mutat Res* **482**:27–40.

Glatt H, Pabel U, Meinl W, Frederiksen H, Frandsen H, and Muckel E (2004) Bioactivation of the heterocyclic aromatic amine 2-amino-3-methyl-9H-pyrido [2,3-b]indole (MeAalphaC) in recombinant test systems expressing human xenobiotic-metabolizing enzymes. *Carcinogenesis* **25**:801–807.

Glatt H, Pauly K, Czich A, Falany JL, and Falany CN (1995) Activation of benzylic alcohols to mutagens by rat and human sulfotransferases expressed in Escherichia coli. *Eur J Pharmacol* **293**:173–181.

Gupta RA, Brockman JA, Sarraf P, Willson TM, and DuBois RN (2001) Target genes of peroxisome proliferator-activated receptor gamma in colorectal cancer cells. *J Biol Chem* **276**:29681–29687.

Gupta RA, Sarraf P, Brockman JA, Shappell SB, Raftery LA, Willson TM, and DuBois RN (2003) Peroxisome proliferator-activated receptor gamma and transforming growth factor-beta pathways inhibit intestinal epithelial cell growth by regulating levels of TSC-22. *J Biol Chem* **278**:7431–7438.

Her C, Kaur GP, Athwal RS, and Weinshilboum RM (1997) Human sulfotransferase SULT1C1: cDNA cloning, tissue-specific expression, and chromosomal localization. *Genomics* **41**:467–470.

Honma W, Shimada M, Sasano H, Ozawa S, Miyata M, Nagata K, Ikeda T, and Yamazoe Y (2002) Phenol sulfotransferase, ST1A3, as the main enzyme catalyzing sulfation of troglitazone in human liver. *Drug Metab Dispos* **30**:944–949.

Huin C, Corriveau L, Bianchi A, Keller JM, Collet P, Krémarik-Bouillaud P, Domenjoud L, Bécuwe P, Schohn H, Ménard D, et al. (2000) Differential expression of peroxisome proliferator-activated receptors (PPARs) in the developing human fetal digestive tract. *J Histochem Cytochem* **48**:603–611.

Jiang YJ, Kim P, Elias PM, and Feingold KR (2005) LXR and PPAR activators stimulate cholesterol sulfotransferase type 2 isoform 1b in human keratinocytes. *J Lipid Res* **46**:2657–2666.

Kocarek TA and Mercer-Haines NA (2002) Squalestatin 1-inducible expression of rat CYP2B: evidence that an endogenous isoprenoid is an activator of the constitutive androstane receptor. *Mol Pharmacol* **62**:1177–1186.

Kodama S, Hosseinpour F, Goldstein JA, and Negishi M (2011) Liganded pregnane X receptor represses the human sulfotransferase SULT1E1 promoter through disrupting its chromatin structure. *Nucleic Acids Res* **39**:8392–8403.

Meinl W, Donath C, Schneider H, Sommer Y, and Glatt H (2008a) SULT1C3, an orphan sequence of the human genome, encodes an enzyme activating various promutagens. *Food Chem Toxicol* **46**:1249–1256.

Meinl W, Ebert B, Glatt H, and Lampen A (2008b) Sulfotransferase forms expressed in human intestinal Caco-2 and TC7 cells at varying stages of differentiation and role in benzo[a]pyrene metabolism. *Drug Metab Dispos* **36**:276–283.

Quandt K, Frech K, Karas H, Wingender E, and Werner T (1995) MatInd and MatInspector: new fast and versatile tools for detection of consensus matches in nucleotide sequence data. *Nucleic Acids Res* **23**:4878–4884.

Richard K, Hume R, Kaptein E, Stanley EL, Visser TJ, and Coughtrie MW (2001) Sulfation of thyroid hormone and dopamine during human development: ontogeny of phenol sulfotransferases and arylsulfatase in liver, lung, and brain. *J Clin Endocrinol Metab* **86**:2734–2742.

Riches Z, Stanley EL, Bloomer JC, and Coughtrie MW (2009) Quantitative evaluation of the expression and activity of five major sulfotransferases (SULTs) in human tissues: the SULT "pie". *Drug Metab Dispos* **37**:2255–2261.

Rondini EA, Fang H, Runge-Morris M, and Kocarek TA (2014) Regulation of human cytosolic sulfotransferases 1C2 and 1C3 by nuclear signaling pathways in LS180 colorectal adenocarcinoma cells. *Drug Metab Dispos* **42**:361–368.

Sakakibara Y, Yanagisawa K, Katafuchi J, Ringer DP, Takami Y, Nakayama T, Suiko M, and Liu MC (1998) Molecular cloning, expression, and characterization of novel human SULT1C sulfotransferases that catalyze the sulfonation of N-hydroxy-2-acetylaminofluorene. *J Biol Chem* **273**:33929–33935.

Sarraf P, Mueller E, Jones D, King FJ, DeAngelo DJ, Partridge JB, Holden SA, Chen LB, Singer S, Fletcher C, et al. (1998) Differentiation and reversal of malignant changes in colon cancer through PPARgamma. *Nat Med* **4**:1046–1052.

Su W, Bush CR, Necela BM, Calcagno SR, Murray NR, Fields AP, and Thompson EA (2007) Differential expression, distribution, and function of PPAR-gamma in the proximal and distal colon. *Physiol Genomics* **30**:342–353.

Teubner W, Meinl W, Florian S, Kretzschmar M, and Glatt H (2007) Identification and localization of soluble sulfotransferases in the human gastrointestinal tract. *Biochem J* **404**:207–215.

Yamazoe Y, Nagata K, Ozawa S, Gong DW, and Kato R (1995) Activation and detoxication of carcinogenic arylamines by sulfation. *Princess Takamatsu Symp* **23**: 154–162.

Zhang L, Huang M, Blair IA, and Penning TM (2012) Detoxication of benzo[a]pyrene-7,8-dione by sulfotransferases (SULTs) in human lung cells. *J Biol Chem* **287**: 29909–29920.

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