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Research Paper

Characterization of Human OATP2B1 (*SLCO2B1*) Gene Promoter Regulation

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Purpose. We investigated transcriptional regulation of organic anion transporter OATP2B1 (*SLCO2B1*) that is expressed in multiple tissues such as liver, small intestine, and others and compared it with that of liver-specific OATPs.
Methods. The promoter activity was examined by luciferase assay. Specific bindings of transcription factors to the promoter region were examined by gel mobility shift assay using native and mutated nucleotides of the promoter region of OATP2B1.
Results. Deletion–mutation study of the promoter region of OATP2B1 showed that the −59 region that included the Sp1 binding site had basal promoter activity, whereas promoter activities of the further upper region were different between intestine-derived Caco-2 cells and liver-derived HepG2 cells. The association of Sp1 to the promoter region was confirmed by gel shift assay and overexpression of Sp1 in cultured cells. Although the promoter of OATP2B1 has a putative HNF1α binding site, overexpression of HNF1α did not induce the expression of OATP2B1.
Conclusion. Sp1, a transcription factor, was required for constitutive expression of OATP2B1 in liver and small intestine, whereas HNF1α, which is involved in the expression of liver-specific OATPs, did not seem to play a role in OATP2B1 expression. Accordingly, it was suggested that the tissue expression profile of OATP2B1 was different from that of other liver-specific OATPs.

KEY WORDS: liver; OATP2B1; promoter; small intestine; transcription factor.

INTRODUCTION

Members of the organic anion-transporting polypeptide (OATP) family are involved in the transport of various endogenous and xenobiotic compounds, such as conjugated metabolites of steroid hormones, thyroid hormones, bile acids, bilirubin, pravastatin, benzylpenicillin, and digoxin (1). Different OATPs have partially overlapping substrate preferences for organic solutes. Some OATPs are preferentially or even selectively expressed in a single tissue, such as liver, whereas others are expressed in multiple tissues. In human liver, OATP2B1 (*SLCO2B1*), OATP1B1 (*SLCO1B1*), and OATP1B3 (*SLCO1B3*) are expressed at the basolateral membrane (2,3). Among them, OATP1B1 and OATP1B3 exhibit specific expression in liver, whereas OATP2B1 is expressed in several tissues, including the intestine, kidney, and liver (1,3). Compared with OATP1B1 and OATP1B3, which accept various anionic compounds as substrates, OATP2B1 has a narrow substrate specificity. Besides transporting the physiological compounds estrone-3-sulfate and dehydroepiandrosterone sulfate, it also mediates the uptake of the xenobiotic sulfobromophthalein (1,3). Because substrates of OATP2B1 overlap with those of OATP1B1 and OATP1B3, the role of OATP2B1 in liver is currently uncertain. Furthermore, it is known that mRNAs of OATP2B1, OATP3A1 (*SLCO3A1*), and OATP4A1 (*SLCO4A1*) are expressed in human intestine (1). We reported that OATP2B1 might play a role in the pH-dependent intestinal absorption of anionic drugs across the apical membrane of human intestinal epithelial cells (4,5). Moreover, we found several genetic polymorphisms of OATP2B1, and one of the single nucleotide polymorphisms of OATP2B1, which has an allelic frequency of about 10% in the Japanese population, showed a decrease in activity in an *in vitro* transport assay using transfected cells (6). Accordingly, it is also important to investigate the expression mechanisms of OATP2B1 in detail to establish the role of OATP2B1 in the small intestine and liver.

At present, information on transcriptional regulation of OATPs is very limited. Hepatic expression of human OATP1B1 and OATP1B3 is dependent on the liver-enriched transcription factor HNF1α (7). The regulatory mechanisms and function of OATP1A2 (*SLCO1A2*) were investigated (8,9). Because OATP1A2 expression was up-regulated by bile acids in patients with primary sclerosing cholangitis (9), it is possible that OATP1A2 plays an important role in the liver. In this study, we investigated transcriptional regulation of OATP2B1, which is expressed in multiple tissues,

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ABBREVIATIONS: DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; MEM, Eagle's minimum essential medium; OATP, organic anion-transporting peptide; PBS, phosphate-buffered saline.

including the liver and small intestine, and is localized at the basolateral membrane of hepatic parenchymal cells, similar to the liver-specific OATP1B1 and OATP1B3.

MATERIALS AND METHODS

Materials

[γ-^{32}P]Adenosine triphosphate (3000 Ci/mmol) was purchased from Amersham Pharmacia Biotech (Piscataway, NJ, USA). Sp1 (PEP2) antibody was purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA). All other reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Wako Pure Chemicals (Osaka, Japan).

Cell Lines and Reagents

The testicular Sertoli cell line (TM4) was purchased from American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured in 50% Dulbecco's modified Eagle's medium (DMEM, Invitrogen, Carlsbad, CA, USA) and 50% F-12 medium (F12, ICN, Biomedicals Inc., Irvine, CA, USA) containing 2.5% fetal bovine serum (FBS, Invitrogen), 5% horse serum, 14 mM $NaHCO_3$, and 15 mM HEPES in a humidified incubator at 37°C under 5% CO_2. HepG2 cells and Caco-2 cells were also obtained from ATCC. HepG2 cells were cultured in Eagle's minimum essential medium (MEM, ICN) containing 2 g/L $NaHCO_3$, 0.1 mM nonessential amino acids (Invitrogen), and 10% FBS in a humidified incubator at 37°C under 5% CO_2. Caco-2 cells were cultured in DMEM containing 2 g/L $NaHCO_3$, 1×10^5 U/L penicillin G, 100 μg/L streptomycin, 1 mM L-glutamine, 0.1 mM nonessential amino acids, and 10% FBS in a humidified incubator at 37°C under 5% CO_2. LS-180 cells were obtained from Dainippon Pharmaceutical Co. (Osaka, Japan). Cells were grown on culture dishes at 37°C in a humidified atmosphere with 5% CO_2. The culture medium consisted of MEM with 10% FBS.

Identification of the OATP2B1 Transcription Start Site

Determination of the transcription start site of the OATP2B1 gene was performed by the CapSite hunting method (10) in accordance with the manufacturer's protocol (Nippon Gene, Tokyo, Japan). Human kidney cap-site cDNA dT, obtained after removal of the 5′ terminal m7 GpppN cap structure of mRNA, was recapped with the 3′ end of a specific rOligo primer provided by the company, and then subjected to nested PCR. The first-round PCR was performed using the rOligo-specific primer 1RDT and OATP2B1-gene-specific primer TGP1 (Table I). The second-round PCR was performed using the rOligo-specific primer 2RDT and OATP2B1-gene-specific primer TGP2. The reactions were performed under the following conditions: at 95°C for 5 min and then 35 or 25 cycles at 95°C for 20 s, at 60°C for 20 s, and at 72°C for 5 min. The PCR product of about 0.30 kb obtained (Fig. 1) was subcloned into pGEM-T easy vector (Promega, San Luis Obispo, CA, USA) and sequenced.

Table I. Sequence of Oligonucleotides Used for Chimeric Plasmid Construction, PCR, and Gel Mobility Shift Assays

Oligonucleotide	Sequence (5′ to 3′)
OATP2B1	
p-2968	CCGACGCGTGCAGACTGTGCTAGG
p-1882	CAAACGCGTGCCAGGTGTGGTGGC
p-1372	AGTACGCGTCCTTTCTCAGTGGAA
p-961	AGTACGCGTGACTCCATGCCTCAT
p-299	CCTACGCGTGGAGGGCAACAAAAG
p-160	CATACGCGTTGTGCCCCCACCTC
p-59	AGCACGCGTGAGGCGGGGTGCAGCT
p-41	TGCACGCGTCTCTTCACAGAGAGG
p-15	GGAACGCGTCAAACCCAGCCTTC
p+192	GGTCTCGAGCAGCAGGGATGCAAT
OATP1B1	
p-424	TTATTCCAGGAGCTCTCTCCAGTCTCC
p+21	GAATCCTAGATCTATGCAACAAGTCCACC
For cap hunting	
1RDT	GAGCTAGCTGCGAGTCAAGTC
2RDT	CGAGTCAAGTCGACGAAGTGC
TGP1	GCCCATTGTGGCTTTGGTTTC
TGP2	GGTCCCATGACTGCTGGAGTG
TGP-sense	AGTGACCCAGGGAGACAAAC
For cloning	
HNF1α sense	TACCAACCAAGAAGGGGCGGA
HNF1α antisense	TCTAGATTTCTGATGCATCAGAGCAGAG
Sp1 consensus sequence	
Wt Sp1(−)	TCAGGGAGGCGGGGTGCAGC
Per Sp1	ATTCGATCGGGGCGGGGCGAGC

Cloning of Human OATP2B1 Promoter

The 5′ region of the OATP2B1 gene was PCR amplified using human genomic DNA (BD Biosciences, Palo Alto, CA, USA) as a template, with upstream primer p-2968, downstream primer p+192 (both synthesized by Hokkaido System Science, Sapporo, Japan; see Table I) and Ex Taq DNA polymerase (Takara Bio Inc., Shiga, Japan), based on the reported OATP2B1 gene sequence (GenBank accession no. AB220159). Because the upstream and downstream primers are designed to include an internal *Mlu*I restriction site and an internal *Xho*I site, respectively, the resulting PCR products were digested with *Mlu*I and *Xho*I and ligated into the luciferase reporter gene vector pGL3-Basic (Promega). The 5′ region of the OATP1B1 gene was PCR amplified using human genomic DNA (BD Biosciences) as a template with upstream primer p-424 and downstream primer p+21 (see Table I; both synthesized by Hokkaido System Science), and Ex Taq DNA polymerase (Takara Bio Inc.) based on the reported OATP1B1 gene sequence (7).

Gel Mobility Shift Assay

Nuclear extracts were prepared from HepG2 and Caco-2 cells as described previously (11). Protein concentration was determined by means of the Bradford method using a protein assay kit (Bio-Rad, Hercules, CA, USA). Oligonucleotides to

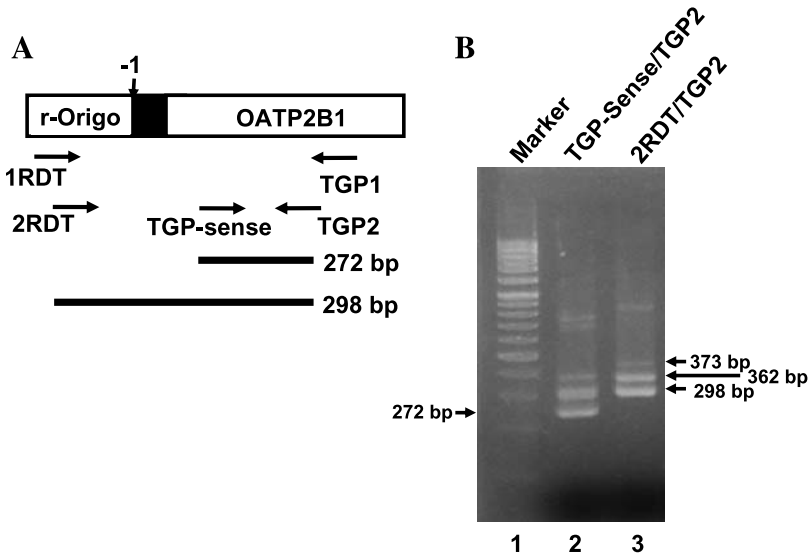

Fig. 1. Identification of the cap site by nested PCR for the determination of the transcription start site of OATP2B1. Cap-site cDNA from human kidney was subjected to nested PCR. The first-round PCR was performed using the rOligo-specific primer 1RDT and OATP2B1 gene-specific primer TGP1, whose positions are illustrated in (A). The second-round PCR was performed using the rOligo-specific primer 2RDT and OATP2B1 gene-specific primer TGP2, whose positions are also illustrated in (A). The resulting product (lane 3) was analyzed by 2% agarose gel electrophoresis and visualized by ethidium bromide staining (B).

the sense and antisense strands containing putative Sp1 binding sites were synthesized (Hokkaido System Science). Their sequences are shown in Table I and mapped onto the OATP2B1 promoter sequence in Fig. 2. Gel mobility shift assays were carried out as described previously (11).

Transfections and Luciferase Assay

Reporter gene constructs were transfected using Lipofectamine 2000 (Invitrogen), according to the manufacturer's protocol. Briefly, cells were plated onto 24-well plates at approximately 0.5×10^5 cells per well for 24 h before transfection. Before addition of DNA/liposome complexes, cells were rinsed with serum-free medium. For each transfection, reporter constructs (0.8 μg) were cotransfected with 0.08 μg of pRL-TK vector (Promega) as an internal control in 0.5 mL of serum-free medium at 37°C for 6 h. After 6 h, the culture medium was changed to medium containing 10% FBS, and the cells were incubated for 48 h at 37°C. Then, the cells were rinsed twice with phosphate-buffered saline (PBS) and harvested using passive lysis buffer (Promega). For luciferase assays, cell extracts were mixed with luciferase assay reagent (Promega) for detection in a luminometer (Berthold GmbH and Co. KG, Germany). Relative luciferase activities are shown as the ratio of firefly/*Renilla* luciferase activities and data are presented as the mean ± SEM of three to ten independent transfection experiments. Sp1 expression vector was a gift from Dr. Alexandrea Stewart (12). HNF1α expression vector was prepared by PCR amplification using Human Liver Marathon-Ready cDNA (BD Biosciences) as a template with upstream primer 5′-TACCAACCAAGAAGGGGC GGA-3′ and downstream primer 5′-TCTAGATTTCTGAT GCATCAGAGCAGAG-3′ (both synthesized by Hokkaido System Science) and Ex Taq DNA polymerase, based on the reported HNF1α gene sequence (13). Upstream and downstream primers were designed to include an internal *Eco*RI restriction site and an internal *Hin*dIII site, respectively, and the resulting PCR products were digested with these enzymes and ligated into expression gene vector pcDNA3.1 (Invitrogen).

RESULTS

Localization of the Transcriptional Initiation Site and Promoter Region of the OATP2B1 Gene

To localize the promoter region of the OATP2B1 gene, the transcription initiation site was identified by cap-hunting methods. A downstream oligonucleotide (Table I) that corresponds to nucleotides +254 to +274 of the OATP2B1 cDNA sequence, as reported by Tamai *et al.* (1) (GenBank accession no. AB026256), afforded three major PCR products, which were amplified and sequenced (Fig. 1). The transcription start site was determined by identification of the boundary between the rOligo and OATP2B1 mRNA sequences. The sequences and 5′ ends were identical. The longest PCR product was 373 bp (from primer pair 1RDT and TGP1), the medium-sized product was 362 bp (from primer pair 1RDT and TGP2), and the short product was 298 bp (from primer pair 2RDT and TGP2) (Fig. 1). Therefore, we assigned the major transcription initiation site of the OATP2B1 gene as +1 in Fig. 2.

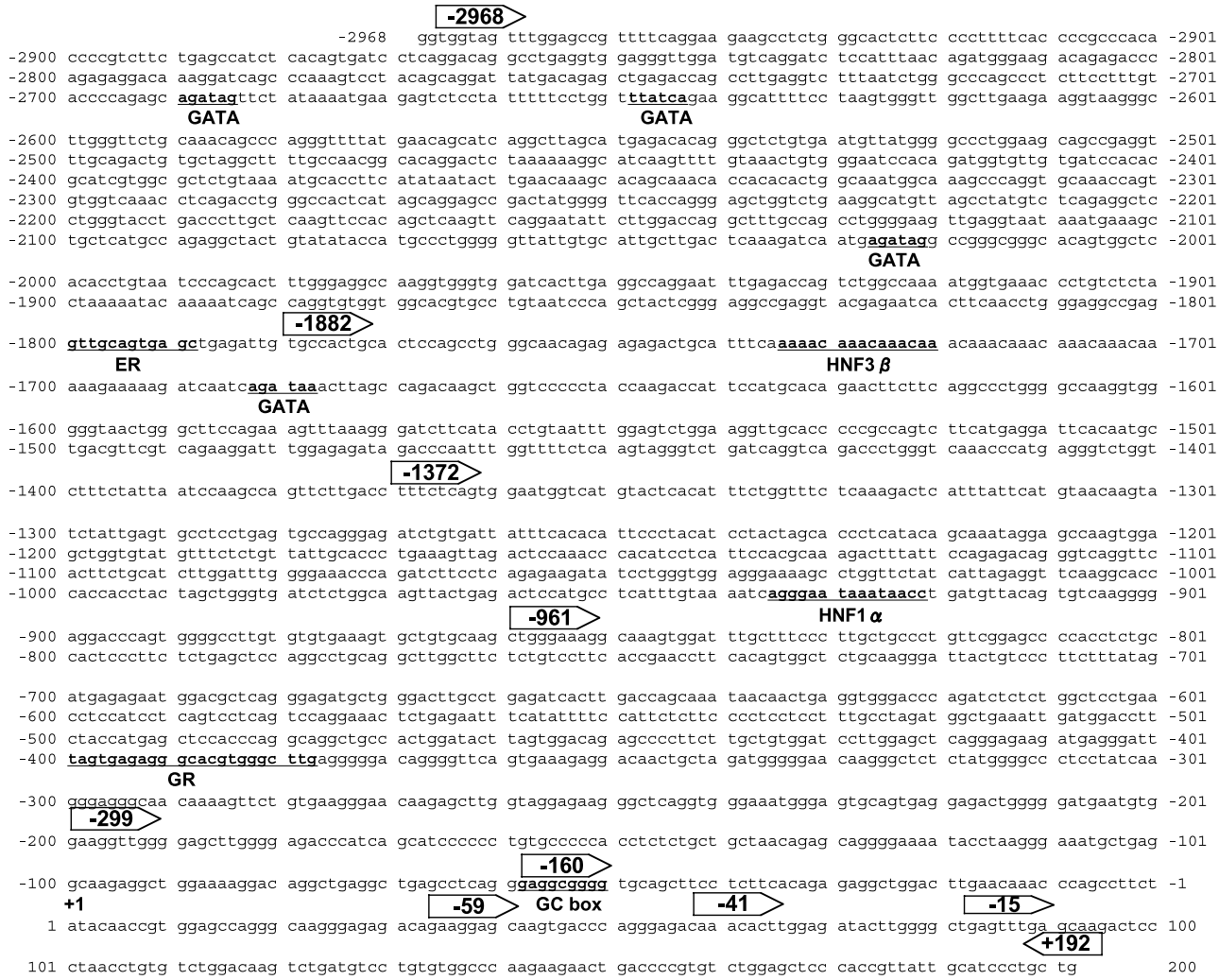

Fig. 2. Nucleotide sequence and structure of the 5′ flanking region of the OATP2B1 gene. Nucleotide sequence of the 5′ flanking region of the human OATP2B1 gene from −2968 to −1 nt. Nucleotide numbers are relative to the transcription initiation site. Consensus binding sites for putative regulatory elements are underlined, and the respective transcription factors are given below the sequence. Flags denote the size of the deletional constructs used for functional promoter studies.

Analysis of the 5′ Flanking Region of the Human OATP2B1 Gene

Base on this finding, the upstream genomic region of the OATP2B1 was identified using the Human Genome Resource of NCBI and NIH. A 2968-bp fragment of the 5′ flanking sequence of the OATP2B1 gene was obtained by PCR amplification (Fig. 2). PCR was performed using human genomic DNA as a template with primers p-2968 and p+192 (Table I). Figure 2 shows the sequence of the 5′ flanking 2968 nucleotides relative to the transcription initiation site up to the translation start site. Potential transcription factor recognition sites were identified by using the program TRANSFAC 4.0 (BIOBASE GmbH, Germany) and the results revealed that this region lacks canonical TATA and CAAT boxes but contains several putative transcription factor binding sites, such as the GC box at −59 to −50, GR at −400 to −377, HNF1α at −936 to −921, GATA at −1683 to −1677, −2036 to −2031, −2659 to −2654, −2689 to −2685, ER at −1800 to −1788, and HNF3β at −1745 to −1730.

Constitutive Activity of the OATP2B1 Enhancer–Promoter Region in Caco-2, LS-180, and HepG2 Cells

To functionally characterize the OATP2B1 promoter, a series of promoter deletions was constructed in reporter gene vector pGL3-Basic, which expresses firefly luciferase. Transfection of the longest p-2968 Luc plasmid into Caco-2, LS180, and HepG2 cells that contained the promoter region from −2968 to −1 nt stimulated luciferase activity about 3-, 5-, and 3-fold, respectively, compared with the pGL3-Basic with no promoter insert (Fig. 3). Promoter activities of all deletion constructs between p-2968 Luc and p-15 Luc were compared with that of pGL3-Basic (Fig. 3). In Caco-2 cells, the constructs p1882, p-1372, p-961, p-299, p-160, and p-59 Luc stimulated luciferase activity 3- to 5-fold compared with pGL3-Basic. p-41 Luc stimulated luciferase activity 2-fold compared with pGL3-Basic, whereas p-15 Luc did not stimulate it (Fig. 3B). In LS-180 cells, the constructs p-1882 and p-41 Luc stimulated luciferase activity about 5-fold, p-1372, p-961, p-299, p-160, and p-59

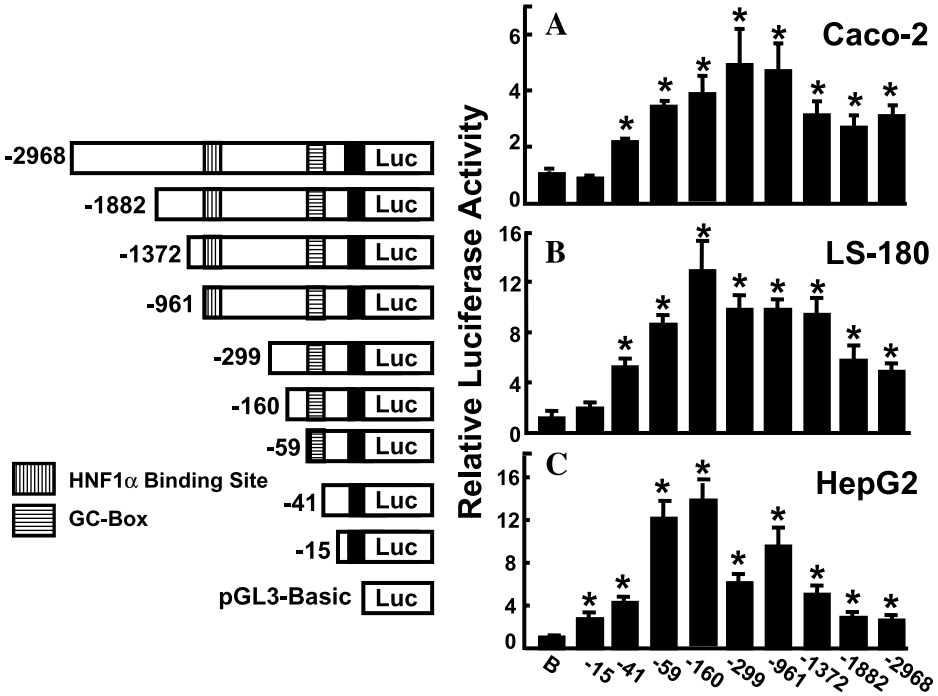

Fig. 3. Analysis of OATP2B1 promoter function in cell lines. Human-intestine-derived cell lines Caco-2 (A) and LS-180 (B), and hepatocyte-derived cell line HepG2 (C) were transiently transfected with chimeric promoter constructs that were inserted into the pGL3-Basic luciferase vector. Transfection efficiency was normalized by cotransfection of pRL-TK, and promoter activity was measured as relative light units of firefly luciferase per unit of *Renilla* luciferase. Promoter activity is shown as the induction factor of luciferase over background activity measured in cells transfected with pGL3-Basic alone (Basic). Results are expressed as the mean ± SEM of five to nine independent transfection experiments. Horizontally striped or vertically striped boxes show putative HNF1α binding sites and GC box, respectively. An asterisk indicates a significant difference ($p < 0.05$) compared with the activity of pGL3-Basic reporter construct.

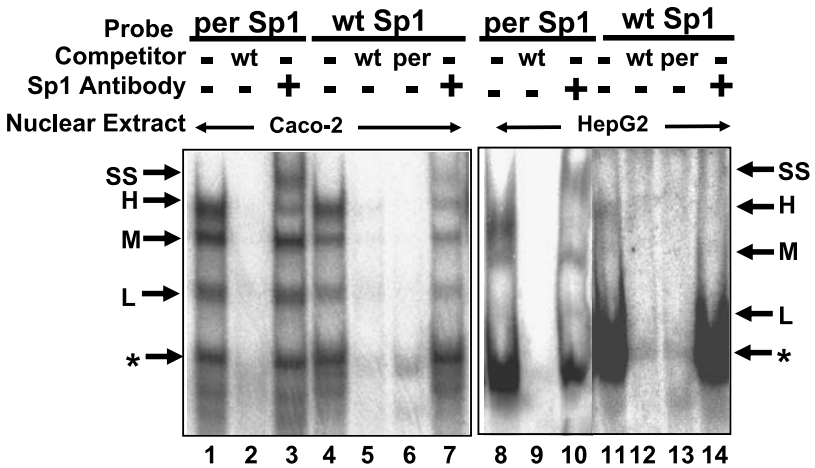

Fig. 4. Gel mobility shift competition assay and supershift analysis of Sp1-bound protein complex. Gel mobility shift assays were carried out with nuclear extract from Caco-2 or HepG2 cells. Lanes 1, 4, 8 and 11, radio-labeled probe alone; lanes 2, 5, 9, and 12, probe with 5 pmol of unlabeled oligonucleotide (wt, self-competition); lanes 6 and 13, probe with 5 pmol of unlabeled oligonucleotide (per, perfect competition); lanes 3, 7, 10 and 14, probe with Sp1-specific antibody (Sp1 Antibody). Four shifted protein complexes are labeled from largest to smallest as H, M, and L and asterisk; the band shown by the asterisk is an unknown complex.

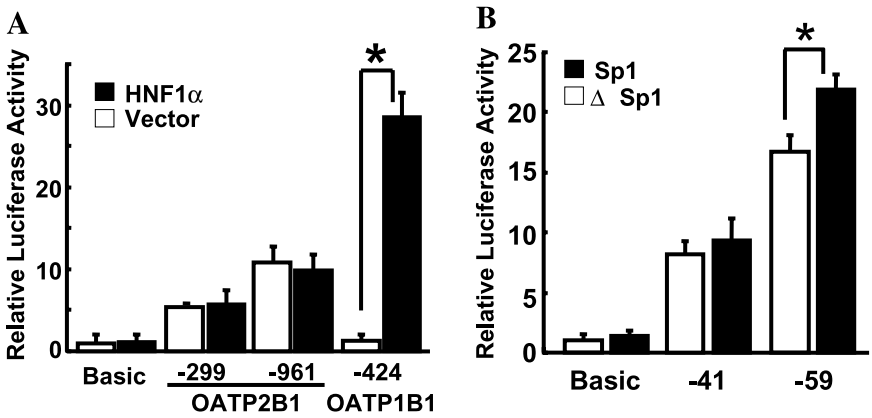

Fig. 5. Effect of HNF1α and Sp1 on OATP2B1 expression. HepG2 cells were cotransfected with the −299 Luc, or −961 Luc reporter gene constructs plus HNF1α (HNF1α, closed bars) or empty vector as a control (open bars) (A). TM4 cells were cotransfected with the −41 Luc or −59 Luc reporter gene constructs plus Sp1 (Sp1, closed bars) or empty vector as a control (open bars) (B).

Luc stimulated it 10-fold, whereas p-15 Luc stimulated it only 2-fold compared with pGL3-Basic (Fig. 3C). In HepG2 cells, the constructs p-1882, p-1372, p-299, p-41, and p-15 Luc stimulated luciferase activity 3- to 5-fold, whereas p-961, p-160, and p-59 Luc stimulated it 10-fold compared with the pGL3-Basic (Fig. 3D). All three cell lines exhibited large increases in promoter activity with p-59 Luc compared with shorter constructs. p-59 Luc includes one GC box that binds transcription factor Sp1 (Fig. 2). Therefore, it was considered that Sp1 controls the basal promoter activity of OATP2B1.

Identification of Putative Sp1 Binding Site in OATP2B1 Promoter Region

To identify nuclear proteins that interact with this *cis*-acting motif, we conducted gel mobility shift assays using nuclear extracts derived from HepG2 and Caco-2 cells and a putative Sp1-binding motif oligonucleotide within human OATP2B1 promoter. The OATP2B1 promoter region from −59 to −1 nucleotides includes one putative Sp1 binding site (Fig. 2). In both Caco-2 and HepG2 cells, three bands of protein complexes, L, M, and H, were observed (Fig. 4, lanes 1, 3, 4, 7, 8, 10, 11, and 14). The protein–DNA complexes shown in Fig. 4 were further analyzed using Sp1-specific oligonucleotides as competitors (Fig. 4, lanes 2, 5, 9, and 12) and antibody directed to Sp1 nuclear protein (Fig. 4, lanes 3, 7, 10, and 14). The shifted complexes were all subject to competition by perfect Sp1 oligonucleotides (Fig. 4, lanes 6 and 13). Furthermore, the shifted complex band H was supershifted by Sp1-specific antibody (Fig. 4, lanes 3, 7, 10, and 14). The signals of band H in lanes 7 and 14 were weaker than those observed without antibody shown in lanes 4 and 11, respectively. The results shown in Fig. 4 demonstrate that the slower migrating protein complex (H) contains Sp1, whereas the two faster migrating (M and L) complexes may contain Sp3 (14). The band below L, indicated by an asterisk in Fig. 4, is an unknown complex, because it was not supershifted by Sp1 antibody. Accordingly, the band below L did not associate with Sp1.

Stimulation of the OATP2B1 Promoter in HepG2 Cells by Overexpression of HNF1α

To identify potential physiological regulators of OATP2B1 expression, we investigated the effect of HNF1α, a transcription factor that regulates hepatocyte-specific expression of OATP1B1 and OATP1B3 (7), on OATP2B1 promoter function. Analysis of potential transcription factor recognition sites revealed a putative HNF1α binding site in the OATP2B1 promoter region (Fig. 2). HepG2 cells were transfected with p-961 and p-299 Luc because p-961 Luc contains a putative HNF1α binding site, whereas p-299 Luc does not (Fig. 2). In addition, we used the promoter of OATP1B1, which is regulated by HNF1α, as a positive control (7). As shown in Fig. 5A, the OATP1B1 promoter was activated by overexpression of HNF1α, whereas the activity of OATP2B1 promoter (p-299 and p-961 Luc) was

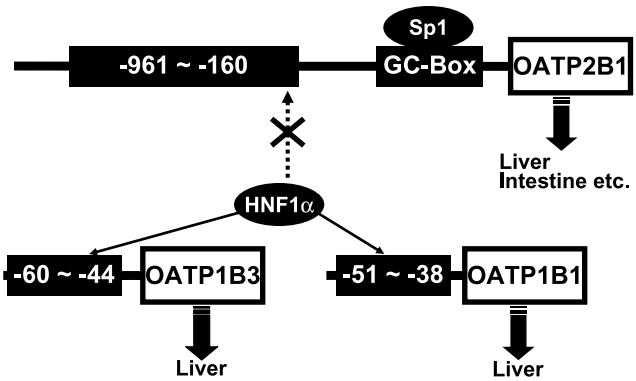

Fig. 6. Schematic model of the OATP2B1 promoter region. Basal promoter activity of OATP2B1 is regulated by Sp1; there is one Sp1 binding site within −59 nt from the transcription initiation site. The region of −961 to −160 might include a negative and activation regulatory region in HepG2 cells and a tissue-specific regulatory region. OATP1B1 and OATP1B3 are regulated by HNF1α, which is not involved in regulation of OATP2B1 expression.

unchanged by overexpression of HNF1α when compared with the transfection of empty vector (Fig. 5A).

Stimulation of the OATP2B1 Promoter in TM4 Cells by Overexpression of Sp1

To investigate whether exogenously expressed Sp1 affects OATP2B1, an expression plasmid coding for Sp1 was introduced into TM4 cells together with two OATP2B1 promoter constructs, one containing a GC box (p-59 Luc) and one without (p-41 Luc). As shown in Fig. 5B, cotransfection of the Sp1-plasmid-enhanced OATP2B1 promoter-driven luciferase activity 1.7-fold (p-59 Luc) compared with cotransfection of empty vector lacking Sp1. However, Sp1 did not activate p-41 Luc and pGL3-Basic (Fig. 5B), indicating that Sp1 binding site expression is important for OATP2B1 basal promoter activity.

DISCUSSION

In the present study, we characterized the promoter region of human OATP2B1, which is expressed in multiple tissues, including the liver and small intestine. The promoter region of OATP2B1 was found to contain consensus recognition sites for both ubiquitously expressed transcription factors, such as Sp1 (12), and hepatocyte-specific transcriptional factor HNF1α (15). The mechanisms of basal promoter activity of OATP2B1 were similar among intestinal-derived cell lines Caco-2 and LS-180 and the hepatocyte-derived cell line HepG2 (Fig. 3). The OATP2B1 promoter region contains one consensus sequence for Sp1 close to the transcription site (Fig. 2), and an examination of basal promoter activity in HepG2, Caco-2, and LS180 cells supported the involvement of Sp1 in the expression of OATP2B1. Binding of Sp1 to this region of the OATP2B1 promoter was confirmed by gel mobility shift assays and supershift analysis using anti-Sp1 antibody (Fig. 4). Coexpression of exogenous Sp1 stimulated OATP2B1 promoter activity 1.5-fold in TM4 cells (Fig. 5) (16). We examined overexpression of Sp1 in HepG2 and Caco-2 cells, but the expression of pRL-TK (encoding *Renilla* luciferase) was affected by Sp1 in HepG2 and Caco-2 cells. Accordingly, these cell lines were unsuitable for studying the effect of Sp1 overexpression, and we used TM4 cells for the analysis. Targeted deletion of the Sp1 binding site (GGGCGG) abolished not only inducibility of the OATP2B1 promoter by Sp1, but even basal promoter function in TM4 cells (Fig. 5B). Others have speculated that Sp1 is a major transcription factor for housekeeping genes, as the Sp1 consensus sequence is commonly found in the promoter region of such genes (17). In addition, synergistic transactivation of mCES2 promoter by Sp1, Sp3, and USF1 was recently reported (18). Accordingly, it is possible that Sp1 interacts with other transcription factors for basic transcriptional regulation of OATP2B1. However, further studies of basal promoter activity of OATP2B1 should be needed. These results suggest that Sp1 controls basal OATP2B1 expression activity, which may be common to multiple tissues.

In HepG2 cells, the sequence from nucleotide −299 to −160 was found to suppress promoter activity (compared with p-160 Luc), and the sequence from nucleotide −961 to −299 activated the OATP2B1 promoter, whereas such changes were not observed in intestinal cell lines (Fig. 3). These results suggested that the −299 to −160 and −961 to −299 regions might include silencer and activation elements, respectively, in the liver, but not in intestine, and that those regions are associated with tissue-specific regulation mechanisms of OATP2B1 expression (Fig. 6). Accordingly, although we could not find *cis* elements corresponding to typical transcription factors in this region of the OATP2B1 promoter, the observations suggest that the expression mechanisms of OATP2B1 differ between liver and intestine. The promoter regulatory mechanisms of Caco-2 cells and LS-180 cells were comparable, but the OATP2B1 promoter activity in LS-180 was higher than that in Caco-2 (Fig. 3). Accordingly, from a quantitative viewpoint, there may be a difference in transcriptional regulation between Caco-2 and LS-180 cells. Indeed, we have reported that Caco-2 cells lack the nuclear receptor SXR (human PXR), whereas it is present in LS180 cells (19). In this experiment, we used three cell lines, HepG2 cells, Caco-2, and LS-180 cells as the models for liver and intestine, respectively, because these cells lines were generally used for analysis of the regulation mechanism of transcriptional expression of genes as a model in respective tissues.

OATP2B1 is widely expressed in various tissues (1). In contrast, OATP1B1 and OATP1B3 are expressed selectively in liver (20,21). The promoters of OATP1B1 and OATP1B3 contain an HNF1α binding site and are regulated by HNF1α (7), whereas the promoter of OATP2B1 was not regulated by HNF1α in this experiment (Fig. 5A). HNF1α is a hepatocyte-specific transcription factor that plays an important role in regulation of the hepatocellular bile acid and organic anion transport systems (13). Mice with null mutations in HNF1α exhibit multiple metabolic abnormalities, including defects in glucose and amino acid homeostasis (22). Loss of HNF1α function in mice also results in hypercholesterolemia, but plasma triglyceride levels are normal. It is thought that HNF1α regulates the expression of proteins that are important in hepatocytes. Therefore, the physiological roles of OATP1B1 and OATP1B3 are liver specific and that of OATP2B1 may be more general in multiple tissues.

Functionally, OATP2B1 exhibits a rather limited substrate specificity compared with OATP1B1 and OATP1B3 at neutral pH (3). However, OATP2B1 shows a broader substrate specificity at acidic pH (4,5). OATP2B1 may play a role in the pH-dependent intestinal absorption of anionic drugs across the apical membrane of human intestinal epithelial cells (4,5). We examined the effect of pH on transcription of OATP2B1 by means of luciferase reporter gene assay and measured the OATP2B1 mRNA levels. However, neither of them was affected by pH (data not shown). When initial uptake of estrone-3-sulfate, a typical substrate of OATP, was studied kinetically, we observed an increase in V_{max} with a decrease of pH from 7.4 to 5.0. We have not examined the expression level of OATP2B1 protein, the stability of OATP2B1, or whether the intrinsic function of OATP2B1 is affected by pH (4).

In conclusion, we analyzed the promoter region of the human OATP2B1 gene and found that the 5′ flanking region contains a *cis*-acting GC box that binds Sp1 and controls basal gene expression in several cell lines from the liver and

intestine. HNF1α, which regulate expression of liver-specific OATP1B1 and OATP1B3, was not involved in the regulation of OATP2B1 (Fig. 6). Accordingly, although these three human OATPs are commonly expressed in liver, their expressional regulation mechanisms are different, suggesting that they have distinct physiological roles.

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