Special Section on Epigenetic Regulation of Drug Metabolizing Enzymes and Transporters

Epigenetic Regulation Is a Crucial Factor in the Repression of UGT1A1 Expression in the Human Kidney

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ABSTRACT

Human uridine 5′-diphospho-glucuronosyltransferase (UGT) 1A1 catalyzes the metabolism of numerous clinically and pharmacologically important compounds, such as bilirubin and SN-38. UGT1A1 is predominantly expressed in the liver and intestine but not in the kidney. The purpose of this study was to uncover the mechanism of the tissue-specific expression of UGT1A1, focusing on its epigenetic regulation. Bisulfite sequence analysis revealed that the CpG-rich region near the *UGT1A1* promoter (-85 to $+40$) was hypermethylated (83%) in the kidney, whereas it was hypomethylated (37%) in the liver. A chromatin immunoprecipitation assay demonstrated that histone H3 near the promoter was hypoacetylated in the kidney but hyperacetylated in the liver; this hyperacetylation was accompanied by the recruitment of hepatocyte nuclear factor (HNF) 1α to the promoter. The *UGT1A1* promoter in human kidney-derived HK-2 cells that do not express UGT1A1 was fully methylated, but this promoter was relatively unmethylated in human liver-derived HuH-7 cells that express UGT1A1. Treatment with 5-aza-2′-deoxycytidine (5-aza-dC), an inhibitor of DNA methylation, resulted in an increase of UGT1A1 mRNA expression in both cell types, but the increase was much larger in HK-2 cells than in HuH-7 cells. The transfection of an HNF1α expression plasmid into the HK-2 cells resulted in an increase of UGT1A1 mRNA only in the presence of 5-aza-dC. In summary, we found that DNA hypermethylation, along with histone hypoacetylation, interferes with the binding of HNF1α, resulting in the defective expression of UGT1A1 in the human kidney. Thus, epigenetic regulation is a crucial determinant of tissue-specific expression of UGT1A1.

Introduction

Uridine 5′-diphospho-glucuronosyltransferases (UGTs) catalyze the glucuronidation of a large number of endogenous and exogenous compounds. In humans, there are 19 functional UGT enzymes; these enzymes are classified into three subfamilies, UGT1A, UGT2A, and UGT2B (Mackenzie et al., 2005). The *UGT1A* genes, located on chromosome 2q37, contain multiple unique first exons and common exons 2 to 5, and they encode nine kinds of functional UGT1A enzymes (Ritter et al., 1992). The *UGT2* genes, located on chromosome 4q13, comprise six exons that are not shared between the UGT2 family members, with the exception of UGT2A1 and UGT2A2, which are formed by exon sharing as in UGT1A. The *UGT2* genes encode three UGT2A and seven UGT2B functional enzymes.

Human UGTs show tissue-specific expression. Although most UGTs are predominantly expressed in the liver, UGT1A7, UGT1A8, and UGT1A10 are exclusively expressed in the gastrointestinal tract (Strassburg et al., 1997, 1998). UGT1A1 is expressed in the liver, small intestine, and colon, but not in the kidney (Nakamura et al.,

2008; Ohno and Nakajin, 2009; Court et al., 2012). The expression of UGT2A1 and 2A2 are limited to the olfactory epithelium (Court et al., 2012). UGT2B7 is abundantly expressed in the liver, kidney, small intestine, and colon, whereas UGT2B10 is expressed only in the liver (Court et al., 2012). To understand the underlying mechanisms of the tissue specific-expression of UGTs, some studies were conducted with a focus on transcriptional regulation (Gardner-Stephen and Mackenzie, 2008; Mackenzie et al., 2010). It has been demonstrated that the intestine-specific transcription factor, caudal-type homeobox protein 2 (Cdx2), as well as Sp1 and hepatocyte nuclear factor (HNF) 1α regulate UGT1A8 and 1A10 expression in the intestine (Gregory et al., 2004). HNF1α and Cdx2 cooperatively regulate UGT2B7 expression in the intestine, whereas HNF1α and octamer transcription factor-1 cooperatively regulate its expression in the liver and kidney (Gregory et al., 2006). HNF1α is also involved in the regulation of UGT1A1 expression in the liver (Bernard et al., 1999). Thus, knowledge of the transcriptional regulation of the tissue-specific expression of the UGTs is accumulating.

However, a question that has yet to be answered is why UGT1A1 is not expressed in the kidney, even though HNF1α is expressed in this tissue (Rey-Campos et al., 1991). In this study, we sought

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ABBREVIATIONS: 5-aza-dC, 5-aza-2′-deoxycytidine; ChIP, chromatin immunoprecipitation; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HNF, hepatocyte nuclear factor; PCR, polymerase chain reaction; TSA, trichostatin A; UGT, uridine 5′-diphospho-glucuronosyltransferase.

to clarify the mechanisms underlying the defective expression of UGT1A1, with a focus on epigenetic regulation. It is known that epigenetic changes, including DNA methylation and histone modification, are key regulators of tissue-dependent gene expression (Shiota, 2004; Ohgane et al., 2008). Supporting our hypothesis, a previous study found that the DNA methylation status of the proximal promoter region of the *UGT1A1* gene affects UGT1A1 expression in colon cancer cell lines (Gagnon et al., 2006). We investigated whether DNA methylation of the promoter and histone modification might be determinants of the tissue-specific expression of human UGT1A1.

Materials and Methods

Materials. 5-Aza-2′-deoxycytidine (5-Aza-dC) and trichostatin A (TSA) were purchased from Sigma-Aldrich (St. Louis, MO). Goat anti-human HNF1α polyclonal antibody (C-19), mouse anti-β-actin monoclonal antibody (C-14), and control rabbit and goat IgGs were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Rabbit anti-human acetyl histone H3 polyclonal antibody was purchased from Millipore (Billerica, MA). Primers were commercially synthesized at Hokkaido System Science (Sapporo, Japan). All other chemicals and solvents were of the highest grade commercially available.

Human Tissues. Human liver and kidney samples from five Japanese donors (donor 1, an 80-year-old woman; donor 2, a 54-year-old man; donor 3, a 39-year-old woman; donor 4, a 13-year-old boy; donor 5, a 40-year-old man) were obtained from autopsy materials that were discarded after pathologic investigation. The use of the human livers and kidneys was approved by the ethics committees of Kanazawa University (Kanazawa, Japan) and Iwate Medical University (Morioka, Japan).

Cell Culture. Human kidney tubular epithelial cell line HK-2 and human hepatocellular carcinoma cell line HuH-7 were obtained from the American Type Culture Collection (Manassas, VA) and the RIKEN BioResource Center (Ibaraki, Japan), respectively. These cells were cultured as previously described (Nakamura et al., 2008).

RNA Isolation and Real-Time Polymerase Chain Reaction. Total RNA was isolated from human liver and kidney samples using RNAiso (Takara Bio, Otsu, Japan) according to the manufacturer's protocol. The cDNA was synthesized from the total RNA using ReverTra Ace (Toyobo, Osaka, Japan). The UGT1A1 mRNA levels were determined by real-time polymerase chain reaction (PCR) and normalized with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA levels as described previously (Izukawa et al., 2009).

Genomic DNA Extraction and Bisulfite Reaction. Genomic DNA samples were prepared from human liver (donor 3) and kidney (donor 1) samples, cell lines, or human hepatocytes (HH268, a 54-year-old Caucasian woman; Tissue Transformation Technologies, Edison, NJ) with a Gentra Puregene Tissue kit (Qiagen, Valencia, CA). Five hundred nanograms of genomic DNA digested with *EcoR* I were treated with bisulfite using the EZ DNA Methylation kit (Zymo Research, Orange, CA). The DNA fragment near the transcription start site of the *UGT1A1* gene was amplified by PCR using the primer pair shown in Table 1. The PCR products were cloned into the pT7Blue T-Vector (Novagen, Madison, WI), and randomly picked clones were sequenced. The DNA methylation status of the sequence was analyzed using the web-based tool QUMA (Kumaki et al., 2008).

Chromatin Immunoprecipitation Assay. The chromatin immunoprecipitation (ChIP) assay was performed using the ChIP assay kit (Millipore) with slight modifications. Approximately 200 mg of frozen human liver (donor 3) or kidney (donor 1) was minced on ice and suspended in 1% (v/v) formaldehyde to cross-link proteins to DNA. After centrifugation, the precipitate was resuspended in cell lysis buffer and homogenized using a Dounce homogenizer. After centrifugation, the precipitate was resuspended in nuclei lysis buffer and sonicated to shear the genomic DNA. After centrifugation, the supernatant (100 μl) was diluted 10-fold with immunoprecipitation dilution buffer and incubated with Dynabeads protein G (Life Technologies, Gaithersburg, MD) conjugated to antibodies against acetylated histone H3 (5 μg) or HNF1α (2 μg). A proportion of the diluted supernatant was kept as an input. The Dynabeads protein G was precipitated and washed sequentially one time each with a low-salt immune complex wash buffer, a high-salt immune complex buffer, and a lithium chloride immune complex buffer. The DNA-protein complex was eluted with elution buffer twice, and the cross-links were reversed by adding sodium chloride. DNA was extracted by phenol-chloroform extraction and ethanol precipitation. The −118 to + 91 region of the *UGT1A1* gene was amplified by real-time PCR with the primers shown in Table 1. The protocol for the PCR was as follows: 95°C for 30 seconds, followed by 45 cycles of 94°C for 4 seconds and 62°C for 20 seconds. DNA extraction and real-time PCR were also performed for the input samples, and the data were used as a control to evaluate the enrichment of DNA in the immunoprecipitates.

Construction of an HNF1α Expression Plasmid. Human HNF1α cDNA was amplified by PCR using the primer pair shown in Table 1 and human liver cDNA as a template. The PCR product was subcloned into the pTARGET vector (Promega, Madison, MI). The nucleotide sequence was confirmed by DNA sequencing analysis.

Chemical Treatment and Transfection of Expression Plasmid into the Cells. HK-2 and HuH-7 cells were seeded onto a 12-well plate at 0.5×10^5 cells/well and incubated for 24 hours. For dose response experiments, the cells were treated with 0.01, 0.1, 1, or 10 μM 5-aza-dC for 120 hours or treated with 50, 100, or 300 nM TSA for 24 hours and then subjected to RNA isolation. For the overexpression of HNF1α, the cells were transiently transfected with 0.5 μg of an HNF1α expression plasmid or an empty pTARGET plasmid using the X-tremeGENE HP DNA transfection reagent (Roche Applied Science, Indianapolis, IN). After 12 hours, the cells were treated with 0.1 μM 5-aza-dC for 96 hours, followed by treatment with TSA for an additional 24 hours. The UGT1A1 mRNA levels were determined as previously described.

Preparation of Nuclear Extract and Immunoblot Analysis of HNF1α. Nuclear extract was prepared from HK-2 and HuH-7 cells transfected with the HNF1α expression plasmid or empty plasmid using NE-PER Nuclear and Cytoplasmic Extraction Reagents (Thermo Fisher Scientific, Rockford, IL) according to the manufacturer's protocols. The protein concentration

TABLE 1

Oligonucleotides used for the UGT1A1 bisulfite analysis and ChIP assay and for the cloning of HNF1α

Nucleotides are numbered with the transcription start site designated as +1 in the *UGT1A1* genomic DNA sequence and base A in the initiation codon ATG designated as +1 in the HNF1α cDNA sequence.

Oligonucleotides	5′ to 3′ Sequence	Position
Bisulfite analysis of UGT1A1		
Forward	TTTGTGGATTGATAGTTTTTTATAG	−113 to −89
Reverse	CAATAACTACCATCCACTAAAATC	+134 to +111
ChIP assay of UGT1A1		
Forward	CTACCTTTGTGGACTGACAGC	−118 to −98
Reverse	CAACAGTATCTTCCCAGCATG	+111 to +91
Cloning of HNF1α		
Forward	GCAGCCGAGCCATGGTTTCT	−11 to +9
Reverse	GGTGCCGTGGTTACTGGGA	+1906 to +1888

ChIP, chromatin immunoprecipitation; HNF, hepatocyte nuclear factor; UGT, uridine 5′-diphospho-glucuronosyltransferase.

was determined using Bradford protein assay reagent (Bio-Rad Laboratories, Hercules, CA) with γ-globulin as a standard. The nuclear extract (40 μg) was separated by 7.5% SDS-PAGE and transferred to an Immobilon-P transfer membrane (Millipore). The membranes were probed with goat anti-human HNF1α or rabbit anti-human GAPDH antibodies followed by fluorescent dye-conjugated second antibodies. The membranes were then scanned using the Odyssey Infrared Imaging system (LI-COR Biosciences, Lincoln, NE).
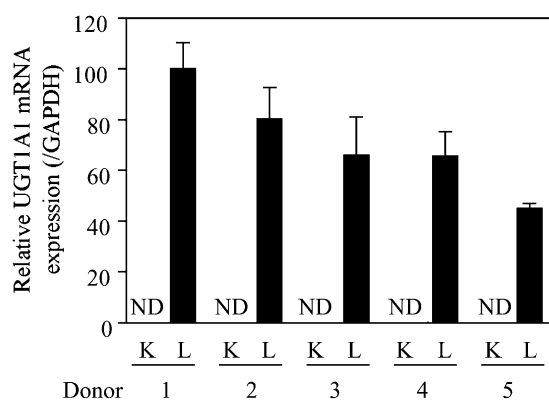
Statistical Analyses. For DNA methylation status, the statistical significance was evaluated by the Mann-Whitney U-test or Fisher's exact test using the web-based tool QUMA. For mRNA expression, statistical significance was determined using an unpaired, two-tailed Student's t test or one-way analysis of variance followed by Dunnett's test. When the P value was less than 0.05, the differences were considered to be statistically significant.

Results

UGT1A1 mRNA Expression in Human Liver and Kidney. UGT1A1 mRNA expression in human liver and kidney was determined by real-time PCR. As shown in Fig. 1, UGT1A1 mRNA was detected in the liver but was negligible in the kidney. The results supported previous studies (Nakamura et al., 2008; Ohno and Nakajin, 2009) that reported the repressed expression of UGT1A1 in the human kidney.
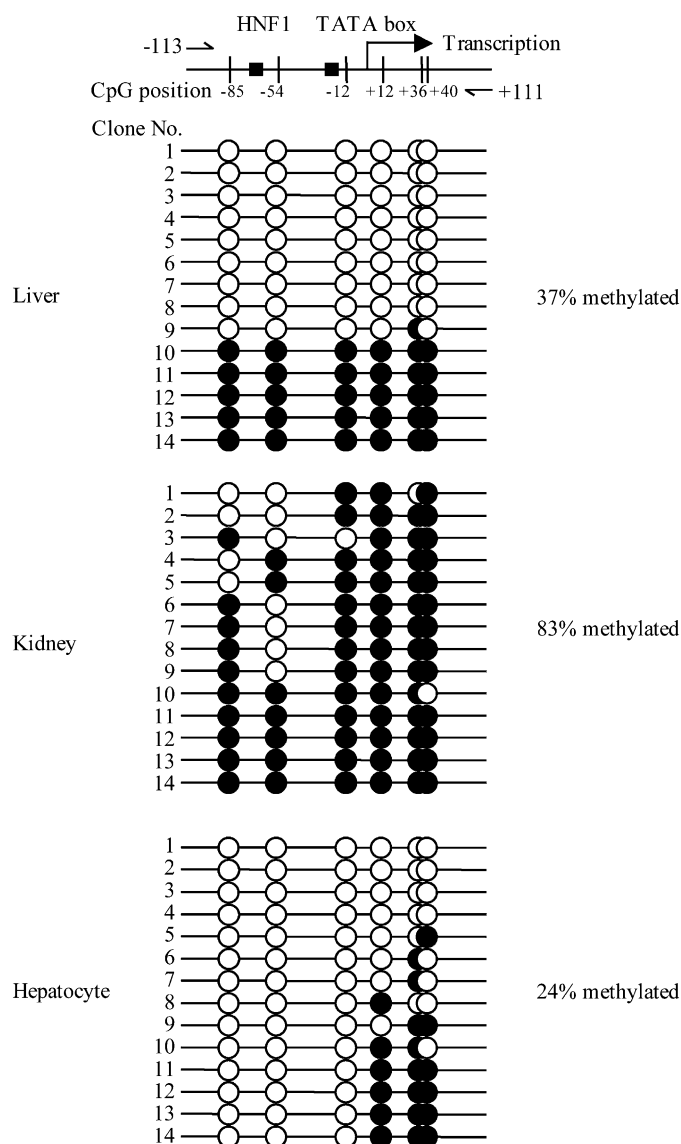
DNA Methylation Status of the *UGT1A1* Promoter Region in Human Liver and Kidney. Genomic DNA extracted from the liver and kidney was treated with bisulfite, and the promoter region of UGT1A1 spanning −113 to +111 was amplified by PCR. The PCR product was subcloned into a vector, and 14 clones from each sample were sequenced. The DNA methylation status of the CpG dinucleotides at −85, −54, −12, +12, +36, and +40 of the *UGT1A1* gene is shown in Fig. 2. In the liver, 31 out of 84 CpG sites (37%) were methylated, whereas in the kidney, 70 out of 84 CpGs (83%) were methylated (P = 0.07, Mann-Whitney U test). Notably, the methylated CpG sites were biased in five clones in the liver. We surmised that these clones might be from hepatic nonparenchymal cells. Hence, we investigated the DNA methylation status of the *UGT1A1* promoter in human hepatocytes and found that the methylated status was only 24% (20 out of 84 CpG sites). In particular, nucleotide positions −85, −54, and −12 were unmethylated in all hepatocyte clones but were hypermethylated in the kidney (P < 0.001; P < 0.01; and P < 0.0001, respectively; Fisher's exact test). Thus, the DNA methylation

status of the UGT1A1 promoter region is different in the liver and kidney.

Histone H3 Acetylation Status and Recruitment of HNF1α to the *UGT1A1* Promoter Region. DNA methylation induces chromatin condensation by recruiting chromatin-remodeling factors, such as methyl-CpG-binding protein and histone deacetylase, thus limiting the access of transcription factors (Bird and Wolffe, 1999). We performed ChIP assays to determine the extent of histone H3 acetylation at the *UGT1A1* promoter in the liver and kidney. In addition, the extent of the recruitment of HNF1α to the *UGT1A1* promoter in the liver and kidney was also determined because it has been demonstrated that HNF1α regulates UGT1A1 expression (Bernard et al., 1999). As

Fig. 2. DNA methylation status of the *UGT1A1* promoter region in human liver, kidney or hepatocytes. Top panel shows a schematic diagram of the *UGT1A1* 5′-flanking region. The vertical lines and numbers represent the position of the cytosine residues of the CpGs relative to the transcription start site as +1. The HNF1 binding site and TATA box are represented by rectangles. Arrows indicate the positions of the primers used for ChIP analysis. Bottom panel shows DNA methylation status of CpG sites. Bisulfite sequencing analysis was performed using genomic DNAs extracted from human liver (donor 3), kidney (donor 1) or hepatocytes (HH268). Fourteen clones from each sample type were sequenced. The open and closed circles represent unmethylated and methylated cytosines, respectively.
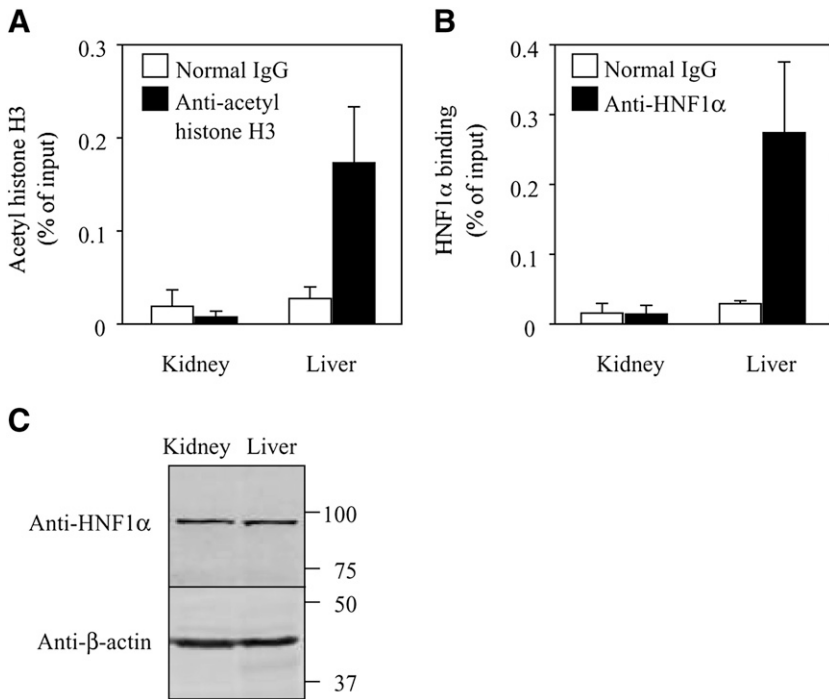

Fig. 1. UGT1A1 mRNA expression in human kidney and liver. The expression levels of UGT1A1 mRNA were determined by real-time PCR and normalized to GAPDH mRNA levels. Each kidney and liver sample with a given number of donors came from the same donors. The values are expressed as relative to the UGT1A1 levels in the liver from donor 1. Each column represents the mean ± S.D. of triplicate determinations. K, kidney; L, liver; ND, not detectable.

Fig. 3. Histone H3 acetylation and recruitment of HNF1α in the *UGT1A1* promoter region in human kidney and liver. (A and B) ChIP assay of acetyl histone H3 and HNF1α in kidney and liver. Human kidney (donor 1) and liver (donor 3) chromatin was precipitated with anti-acetyl histone H3 antibody (A) or anti-HNF1α antibody (B). The precipitated DNA was quantified by real-time PCR with a primer pair that amplified the region from −118 to +111 of the *UGT1A1* gene. The results are expressed as the percentage of input. Normal rabbit or goat IgGs (open columns) were included as negative controls. (C) Western blot analysis of HNF1α in kidney and liver. Homogenates (50 μg) from kidney and liver samples were subjected to 10% SDS-PAGE and probed with anti-HNF1α or anti-β-actin antibodies. Each column represents the mean ± S.D. of triplicate determinations.

shown in Fig. 3A, acetylated histone H3 was enriched at the *UGT1A1* promoter in the liver but not in the kidney. In addition, it was demonstrated that HNF1α was highly recruited to the *UGT1A1* promoter in the liver but not in the kidney (Fig. 3B). Western blot analysis demonstrated that HNF1α is expressed in kidney and liver equally (Fig. 3C). These results suggest that the DNA hypermethylation in the kidney could be linked to abolished histone H3 acetylation and HNF1α binding.

Effects of the Inhibition of DNA Methylation and Histone Deacetylation and the Transfection of Exogenous HNF1α on UGT1A1 Expression. To investigate the significance of the DNA methylation at the promoter region in the repression of UGT1A1 expression, we performed a series of experiments using cell lines. We selected two cell lines, the human kidney-derived HK-2 line and liver-derived HuH-7 cells. We found that the *UGT1A1* promoter region was hypermethylated (98%) in HK-2 cells but was moderately methylated (47%) in HuH-7 cells ($P < 0.0001$, Fig. 4A). UGT1A1 mRNA was marginally expressed in HK-2 cells but was substantially expressed in HuH-7 cells (~4800-fold difference) (Fig. 4B), suggesting that DNA methylation negatively regulates UGT1A1 expression in HK-2 cells. To investigate whether the inhibition of DNA methylation could induce UGT1A1 expression, the cells were treated with 5-aza-dC, an inhibitor of DNA methylation. Although this treatment increased UGT1A1 mRNA in both cell lines, the induction was higher in HK-2 cells (~400-fold at maximum) than in HuH-7 cells (~6-fold at maximum) (Fig. 4B). We confirmed that 5-aza-dC treatment efficiently decreased the methylation status in HK-2 to 33% ($P < 0.001$) and in HuH-7 cells to 7% ($P < 0.001$) (Fig. 4C).

The UGT1A1 mRNA level in HK-2 cells treated with 0.1 μM 5-aza-dC was still low compared with that in HuH-7 cells. We suspected that HNF1α might be lacking in HK-2 cells, thus causing the lower UGT1A1 levels. Western blot analysis demonstrated that HNF1α is expressed at very low levels in HK-2 cells (Fig. 4D). To investigate the significance of the DNA methylation status in the suppression of UGT1A1 expression, we sought to exogenously express HNF1α in HK-2 cells. The HNF1α protein level was dramatically increased by the transfection of the HNF1α expression plasmid into HK-2 cells (Fig. 4D), but UGT1A1 mRNA expression was not increased (Fig. 4E). These results suggested that DNA methylation inhibits the binding of HNF1α to the promoter of UGT1A1. However, under 5-aza-dC treatment, the overexpression of HNF1α resulted in a significant increase of UGT1A1 mRNA expression (4.3-fold) in HK-2 cells. This phenomenon was not observed in HuH-7 cells, implying that endogenous HNF1α expression levels might be sufficient for UGT1A1 in HuH-7 cells (Fig. 4D).

Finally, we investigated whether histone deacetylation is also involved in the repression of UGT1A1 expression. When the HK-2 and HuH-7 cells were treated with TSA, an inhibitor of histone deacetylation, UGT1A1 mRNA expression was unchanged (Fig. 4E). However, TSA treatment facilitated (by 1.7-fold) the increase of UGT1A1 mRNA by 5-aza-dC treatment in HK-2 cells in the presence of exogenously expressed HNF1α. This result was not observed in HuH-7 cells. Collectively, these results suggest that DNA methylation status, and to a lesser extent histone deacetylation status, are critical determinants of UGT1A1 expression.

Discussion

Human UGT1A1 is predominantly expressed in the liver and the intestine but not in the kidney. Previous studies demonstrated that HNF1α and HNF1β are involved in the constitutive (Bernard et al., 1999) and inducible expression of UGT1A1 (Sugatani et al., 2008) by binding to a site approximately 30 bp upstream of the TATA box. The expression of HNF1α and HNF1β is not confined to the liver, as these genes are expressed in various tissues, including the kidney, intestine, stomach, and pancreas (Harries et al., 2006). Therefore, the reason for the repressed expression of UGT1A1 in the kidney remained to be clarified. To uncover the underlying mechanism, we conducted studies focusing on epigenetic regulation. HNF1α and HNF1β form homodimers or heterodimers and equally *trans*-activate the *UGT1A1* gene (Bernard et al., 1999). Therefore, HNF1α was studied as the representative *UGT1A1* activator.
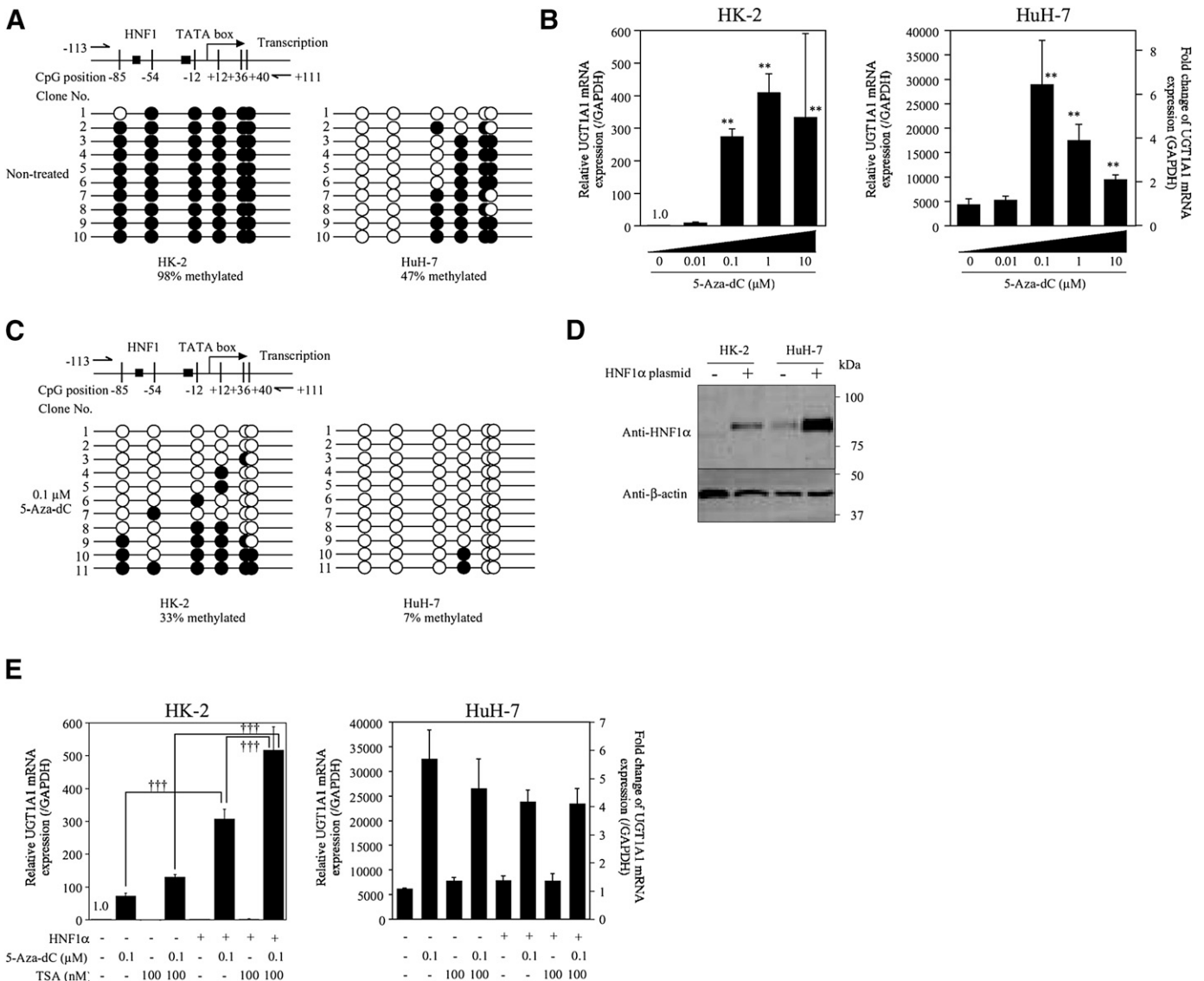
Fig. 4. Effects of 5-aza-dC and/or TSA treatment and transfection of HNF1α on the UGT1A1 expression in HK-2 and HuH-7 cells. (A) DNA methylation status of the *UGT1A1* promoter region in HK-2 and HuH-7 cells. Ten clones each were sequenced. The open and closed circles represent unmethylated and methylated cytosines, respectively. (B) Effects of 5-aza-dC on the UGT1A1 expression in HK-2 and HuH-7 cells. UGT1A1 mRNA level was determined by real-time RT-PCR and normalized with the GAPDH mRNA levels. (C) Effects of 5-aza-dC on the DNA methylation status of the *UGT1A1* promoter region in HK-2 and HuH-7 cells. Bisulfite sequencing analysis was performed using genomic DNA extracted from 5-aza-dC-treated cells. (D) Western blot analysis of HNF1α in HK-2 and HuH-7 cells. Nuclear extracts from HK-2 and HuH-7 cells transfected with HNF1α expression plasmid (+) or empty plasmid (−) were analyzed. (E) Effects of 5-aza-dC and/or TSA treatment and transfection of HNF1α on the UGT1A1 mRNA expression in HK-2 and HuH-7 cells. The cells were transiently transfected with HNF1α expression plasmid (+) or empty plasmid (−), followed by treatment with 5-aza-dC and/or TSA. The expression level of UGT1A1 mRNA was determined by real-time PCR. Data were expressed as relative to UGT1A1 expression compared with nontreated HK-2 cells. Each column represents the mean ± S.D. of triplicate determinations. **$P < 0.01$, compared with nontreated cells; $^{†††}P < 0.001$.

We found that the CpG island at the promoter region of the *UGT1A1* gene in the kidney was hypermethylated, whereas it was hypomethylated in the liver (Fig. 2). Upon DNA methylation, gene silencing occurs by two mechanisms: 1) the methyl group physically interrupts the binding of transcription factors to their recognition sequences, and 2) methyl-CpG-binding proteins bind to the methylated DNA and recruit co-repressor molecules, including histone deacetylase, to induce chromatin structure condensation (Shiota, 2004). Previously, it was demonstrated by gel shift assay that the methylated CpG sites at the *UGT1A1* promoter did not prevent the binding of HNF1α (Bélanger et al., 2010). In contrast, the present study demonstrated that DNA hypermethylation of the *UGT1A1* promoter in the kidney was accompanied by increased acetylation of

histone H3 and defective recruitment of HNF1α (Fig. 3). Therefore, gene silencing of UGT1A1 in the kidney would be due to the latter mechanism with the abolished binding of HNF1α.

Our cell line based study clearly demonstrated the significance of DNA methylation in the regulation of UGT1A1 as follows: 1) substantial expression of UGT1A1 mRNA is observed in HuH-7 cells with DNA hypomethylation status; 2) 5-aza-dC treatment resulted in an increase of UGT1A1 expression that reflected the change in methylation status; and 3) the exogenously expressed HNF1α could increase UGT1A1 expression only in the presence of 5-aza-dC in HK-2 cells. These findings clearly illustrated that unmethylated DNA is a prerequisite for the transcriptional activation of UGT1A1.

The study using TSA demonstrated that histone acetylation is a supplemental factor for transactivation, supporting the general perception (Cameron et al., 1999). In contrast to our study, a previous study reported a significant increase of UGT1A1 mRNA expression following treatment with 3 mM TSA in HepG2 cells (Mackenzie et al., 2010). When we treated the HK-2 and HuH-7 cells with 1 mM TSA, a prominent decrease of cell viability was observed. Thus, it is possible that there are inter-cell line differences in the response toward TSA. Collectively, DNA methylation at the promoter region of UGT1A1 may evoke the condensed chromatin structure through histone deacetylation, thereby inhibiting the binding of such transcription factors as HNF1α. This theory would explain the defective expression of UGT1A1 in kidney, where HNF1α is substantially expressed.

Although the simultaneous overexpression of HNF1α and inhibition of DNA methylation tremendously induced UGT1A1 mRNA in HK-2 cells, the UGT1A1 level was still lower than the level in HuH-7 cells (Fig. 4). It was surmised that some factors regulating UGT1A1 expression might be insufficient in HK-2 cells. Previous studies have reported that pregnane X receptor (Sugatani et al., 2008), glucocorticoid receptor (Usui et al., 2006), constitutive androstane receptor (Sugatani et al., 2008), peroxisome proliferator-activated receptor α (Senekeo-Effenberger et al., 2007), NF-E2-related factor-2 (Yueh and Tukey, 2007), and aryl hydrocarbon receptor (Yueh et al., 2003) are involved in UGT1A1 regulation. It is possible that such factors may be insufficient in HK-2 cells, although experimental proof is required. As another possibility, differences in histone modifications other than acetylation are feasible, namely H3K4 methylation (activating mark), H3K9 methylation (silencing mark), and H3K27 methylation (silencing mark). Thus, such factors might also be involved in the regulation of the basal expression of UGT1A1 in cell lines and tissues.

Each member of UGT1A family has a unique promoter. The tissue-specific expression of UGT1As could be attributed to the differences in their promoter activation (Gong et al., 2001). It is reasonable to assume that UGT isoforms other than UGT1A1 showing tissue-specific expression might also be epigenetically regulated. We are currently working on this issue.

In conclusion, we found that the DNA methylation status of the human *UGT1A1* promoter is different in the liver and kidney. DNA methylation, hypoacetylation of histone H3, and diminished binding of HNF1α could explain the defective expression of UGT1A1 in the kidney. A remaining future challenge is the elucidation of the degree to which UGT1A1 expression is influenced by factors affecting epigenetic status, such as aging, sex, disease, and lifestyle habits.

Acknowledgments

Authorship Contributions

Participated in research design: Oda, Nakajima, Fukami, Yokoi.

Conducted experiments: Oda.

Performed data analysis: Oda.

Wrote or contributed to the writing of the manuscript: Oda, Nakajima, Yokoi.

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