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Research Paper

DNA Methylation Profiles of Organic Anion Transporting Polypeptide 1B3 in Cancer Cell Lines

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Purpose. Multispecific organic anion transporter, OATP1B3/*SLCO1B3*, is expressed in several cancer cell lines as well as tumor tissues, and its expression sensitizes the cells to some anti-cancer agents. The present study was aimed to characterize the DNA methylation profiles around the transcriptional start site (TSS) of OATP1B3 and correlate them with the mRNA expression in cancer and immortalized cell lines.
Methods. The mRNA expression and DNA methylation profiles of OATP1B3 were determined by RT-PCR and bisulfite sequencing, respectively.
Results. The expression of OATP1B3 mRNA was detected in DLD-1, TFK-1, PK-8, and PK-45P cells, but below the limit of detection in HepG2, Caco-2, and HEK293 cells. Bisulfite sequencing demonstrated that CpG dinucleotides around the TSS are differentially methylated among cell lines and partly associated with the mRNA expression profile of OATP1B3. Furthermore, treatment with 5-aza-2′-deoxycytidine, an inhibitor of DNA methyltransferase, significantly increased the mRNA expression of OATP1B3 in HepG2 and Caco-2 cells by 18- and 14-fold, respectively, but not in DLD-1 and TFK-1 cells.
Conclusion. DNA methylation-dependent gene silencing is at least partly involved in the regulation of OATP1B3 expression in cancer/immortalized cell lines.

KEY WORDS: cancer cell line; DNA methylation; epigenetics; OATP1B3; organic anion transporter.

INTRODUCTION

Organic Anion Transporting Polypeptides (OATPs) are expressed in the cellular surface of a variety of organs, where these transporters play important roles in the transmembrane transport of a broad range of endogenous and exogenous compounds. At the moment, several isoforms in the OATP family have been functionally characterized in humans (1). In particular, OATP1B1 and OATP1B3 are predominantly expressed in the liver in normal tissues, and mediate the hepatic uptake of clinically important drugs, such as HMG-CoA reductase inhibitors and angiotensin II receptor antagonists (2–5). OATP1B3 also mediates the cellular uptake of several anticancer drugs, such as methotrexate, paclitaxel, docetaxel, and SN-38, an active metabolite of irinotecan (6–8), prompting researchers to investigate the role of OATP family transporters in anticancer therapy.

Several groups, including ourselves, have investigated the clinical impact of polymorphism of *SLCO1B1* and *SLCO1B3* gene encoding OATP1B1 and OATP1B3, respectively (9). The *SLCO1B1*15* allele (388A>G and 521T>C; N130D and V174A) is generally associated with reduced function both *in vitro* and *in vivo* (10,11). A couple of clinical studies have demonstrated that *SLCO1B1* polymorphism is associated with the pharmacokinetics and side effects of irinotecan. The plasma AUC of irinotecan and its active form, SN-38, is significantly greater in cancer patients harboring the *SLCO1B1*15* haplotype than that with the reference diplotype *SLCO1B1*1a/*1a* (12), and the frequency of grade 4 neutropenia is significantly higher in subjects with *SLCO1B1* 521TC or CC genotypes, while that of grade 3 diarrhea is higher in homozygotes of *SLCO1B1* 388G allele among patients with non-small cell lung cancer (13). In addition, we have recently reported that a single nucleotide polymorphism in the intronic region of the *SLCO1B3* gene is associated with docetaxel-induced leukopenia, although its functional significance needs to be examined (14). These observations suggest the significant contribution of OATP1B1 and OATP1B3 to the disposition of anticancer drugs in the body.

Despite its exclusive expression in the liver in normal tissues, OATP1B3 is also expressed in several cell lines originating from gastric, colon, pancreas and gallbladder

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ABBREVIATIONS: COBRA, combined bisulfite restriction analysis; FXR, farnesoid X receptor; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HNF, hepatocyte nuclear factor; OATP, organic anion transporting polypeptide; OAT, organic anion transporter; TSS, transcriptional start site; URAT1, urate transporter 1; 5azadC, 5-aza-2′-deoxycytidine.

cancer (6) as well as human lung and breast tumor tissues (15,16) at the mRNA and/or protein levels. Abundant expression of OATP1B3 may facilitate the accumulation of anticancer drugs that are substrates of this transporter in cancer cell lines and, thereby, enhance their cytotoxic effects. Indeed, bromosulfophthalein, an inhibitor of OATP1B3, attenuates the cytotoxic effect of methotrexate in KatoIII and PK-8 cells, where OATP1B3 is endogenously expressed, and exogenous expression of OATP1B3 sensitizes MDCK cells 7.5-fold to methotrexate compared with mock cells (6). However, the underlying mechanism of the expression of OATP1B3 in cancer cell lines or tumors remains to be clarified.

Cumulative evidence has demonstrated the involvement of several transcription factors in the transcriptional regulation of OATP1B3. Hepatocyte Nuclear Factor (HNF) 1α and Farnesoid X receptor (FXR) play important roles in the transactivation of OATP1B3 promoter under physiological and cholestatic conditions, respectively, and influence the expression in the liver (17,18). HNF1β may also constitute the regulation of OATP1B3 expression, since it shares the recognition sequence with HNF1α and can activate the same promoter but with lower transactivation potency than HNF1α. HNF3β inactivates the transcription of OATP1B3, providing a mechanism for reduced OATP1B3 expression in hepatocellular carcinomas (19). We have recently demonstrated that the liver- and kidney-specific expression of solute carrier and ATP binding cassette transporters is well associated with the DNA methylation status near the transcriptional start site (TSS) (20–22). DNA methylation is one of the most characterized mechanisms underlying the epigenetic regulation of gene expression. Methylation of cytosine residue in the CpG dinucleotide recruits chromatin remodeling factors, including histone deacetylases, leading to the transcriptional repression of neighboring gene regions (23,24). Besides the significant role under physiological conditions, it is worthwhile to consider the prevalent alterations in DNA methylation profiles during carcinogenesis (25,26).

The present study aimed to investigate the correlation between the mRNA expression of OATP1B3 and DNA methylation status around the TSS in cancer cell lines. The mRNA expression of transcription factors involved in the regulation of OATP1B3 was also determined in order to unveil their contribution in cancer. In addition, we have examined the role of DNA methylation in the transcriptional regulation by monitoring the effect of DNA methylation inhibitor on the mRNA expression of OATP1B3.

MATERIALS AND METHODS

Materials

All reagents were purchased from Wako Pure Chemicals (Osaka, Japan) unless stated otherwise.

Cell Culture

HepG2, Caco-2, and HEK293 cells were maintained as described previously (20). DLD-1, TFK-1, PK-8, and PK-45P cells were kindly provided by the Cell Resource Center for Biomedical Research, Institute of Development, Aging and Cancer, Tohoku University, Japan, and maintained in a culture medium consisting of RPMI1640 medium (Invitrogen, Carlsbad, CA) with 10% fetal bovine serum (Sigma-Aldrich, St. Louis, MO). The origins of the cell lines are as follows: HepG2, hepatocellular carcinoma; Caco-2 and DLD-1, colorectal carcinoma; HEK293, human embryonic kidney; TFK-1, bile duct carcinoma; PK-8 and PK-45P, pancreatic carcinoma.

RNA Isolation and RT-PCR

Total RNA was prepared from cells by a single-step guanidium thiocyanate procedure using ISOGEN (Nippon Gene, Toyama, Japan). The RNA was then reverse-transcribed using a random-nonamer primer (Takara, Shiga, Japan). PCR was performed with the forward and reverse primers listed in Table I to detect the partial fragments of OATP1B3, FXR, HNF1α, HNF1β, HNF3β, and GAPDH cDNA. PCR was performed under the following conditions: 94°C 2 min; 28 cycles (OATP1B3) or 35 cycles (FXR, HNF1α, HNF1β, and HNF3β) of 94°C for 30 s, 58°C for 30 s, and 72°C for 1 min, or 25 cycles (GAPDH) of 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min; final extension 72°C for 5 min.

Bisulfite Reaction

Genomic DNA from each cell line was extracted using a Get Pure DNA Kit (Dojindo Molecular Technologies, Gaithersburg, MD). One to two μg genomic DNA digested with BamHI were subjected to the bisulfite reaction as described previously, and the purified DNA was resuspended in 10 mM Tris-HCl and 1 mM EDTA, pH 8.0 (21).

Combined Bisulfite Restriction Analysis and Bisulfite Genomic Sequencing

The DNA fragments covering the genomic region around TSS of the OATP1B3 gene were amplified by PCR using the following three sets of primers: −762-F and −592-R, −464-F and −303-R, or −22-F and +249-R (Table I). PCR was performed using Immolace DNA Polymerase (Bioline, London, UK) under the following conditions: 94°C for 10 min; 43 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 1 min; final extension 72°C for 10 min. PCR fragments of the OATP1B3 upstream region from −464 to −303 were digested with HpyCH4IV (New England Biolabs, Beverly, MA) to evaluate the methylation status of −423 CpG dinucleotide. Because only unmethylated cytosine residues are changed to thymines by the bisulfite reaction, PCR fragments from unmethylated genomic DNA are resistant to HpyCH4IV digestion, while those from methylated DNA are digested. The resultant products were electrophoresed using 2.0% agarose gel and stained with ethidium bromide. For bisulfite genomic sequencing, the PCR products were cloned into pGEM-T Easy vector (Promega, Madison, WI), and 10 clones randomly picked from each sample were sequenced to determine the presence of methylated cytosines.

5-aza-2′-Deoxycytidine Treatment and Quantification of the mRNA Expression of OATP1B3 and Transcription Factors

Prior to the treatment with 5-aza-2′-deoxycytidine (5azadC) (DNA methylation inhibitor; Sigma-Aldrich),

Table I. Oligonucleotides Used for RT-PCR, Quantitative PCR, and Bisulfite PCR

Origonucleotide	Orientation	Sequence (5′ to 3′)
Primers used for the RT-PCR or the quantitative PCR		
OATP1B3	forward	GGGTGAATGCCCAAGAGATA
	reverse	ATTGACTGGAAACCCATTGC
FXR	forward	CTCTACCAGGGAGAAACTGAG
	reverse	CTCCTGAAGAAACCTTTACACC
HNF1α	forward	TGGGTCCTACGTTCACCAAC
	reverse	TCTGCACAGGTGGCATGAGC
HNF1β	forward	TGCACAAAGCCTCAACACCT
	reverse	TCCACGACGTACTCAGCGC
HNF3β	forward	ATGGAAGGGCACGAGCC
	reverse	TTGCTCACGGAGGAGTAGCC
GAPDH	forward	AATGACCCCTTCATTGAC
	reverse	TCCACGACGTACTCAGCGC
Primers used for the bisulfite PCR		
OATP1B3		
−762-F	forward	GTGTATATATATATGTGAGAGAAGATTT
−592-R	reverse	CCTTCACTCAATTAAAACTTTTCTTAAA
−464-F	forward	GGTAATTTAGTATTTTATGTGAGATATTT
−303-R	reverse	CTATTCCAAATAAAAAATATATCAAAA
−22-F	forward	AAGTTAATTAATTATTTAAAGTGAGAT
+249-R	reverse	CTAAAACTCTTCTATTTCTAAAAAAA

HepG2, Caco-2, DLD-1, and TFK-1 cells were plated in 12-well plates at the density of 1.0×10^5 cells/well and precultured for 24 h. Then, cells were cultured for 72 h in medium containing 0, 1, 10, or 100 μM 5azadC. To quantify the mRNA expression of OATP1B3, FXR, HNF1α, HNF1β, HNF3β, and GAPDH, real-time quantitative PCR was performed using a LightCycler™ and the appropriate software (version 3.53; Roche Diagnostics) according to the manufacturer's instructions. cDNA used for the quantification was prepared as described above. Primers used in this study are shown in Table I. PCR was performed using a SYBR Premix Ex Taq (perfect real time) (Takara). The protocol for PCR was as follows: 95°C for 30 s; 40 cycles of 95°C for 5 s, 58°C (OATP1B3) or 55°C (FXR, HNF1α, HNF1β, HNF3β, and GAPDH) for 10 s, and 72°C for 15 s. A standard curve was generated by dilutions of the target PCR product, which had been purified and had its concentration measured. To confirm the amplification specificity, the PCR products were subjected to a melting curve analysis. The fold induction in the mRNA expression by 5azadC treatment was calculated compared with the untreated cells in each cell line.

RESULTS

mRNA Expression of OATP1B3 and Transcription Factors in Human Cancer Cell Lines

The mRNA expression of OATP1B3 was investigated in HepG2, Caco-2, HEK293, DLD-1, TFK-1, PK-8, and PK-45P cells by RT-PCR (Fig. 1). The expression of OATP1B3 mRNA was below the limit of detection in HepG2, Caco-2, and HEK293 cells, while significant amplification was observed in DLD-1, TFK-1, PK-8, and PK-45P cells. These observations were in good agreement with the previous report (6). The seven cell lines were used in a further analysis on the basis of the presence or absence of OATP1B3 mRNA in order to describe the role of DNA methylation and transcription factor network in the expression of OATP1B3 in cancer cell lines. We have examined the mRNA expression of FXR, HNF1α, HNF1β, and HNF3β involved in the transcriptional regulation of OATP1B3. The mRNA expression of OATP1B3 transactivators, FXR, HNF1α, and HNF1β, were detected even in OATP1B3-negative cell lines (HepG2 and Caco-2), and that of the OATP1B3 suppressor, HNF3β, was detected at a similar level in all cell lines tested.

DNA Methylation Status of −423 CpG Dinucleotide in the Upstream Region of OATP1B3 Gene

The DNA methylation status of the CpG dinucleotide located at −423 bp relative to the TSS of OATP1B3 was

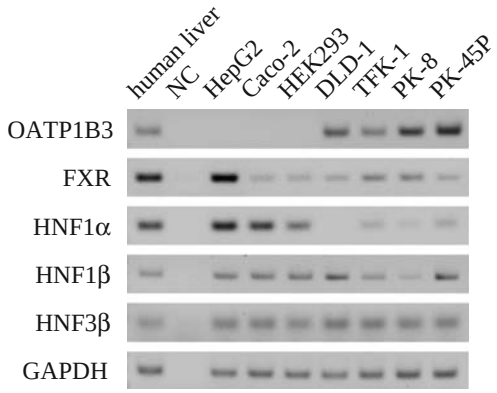

Fig. 1. Expression of OATP1B3 and transcription factors in cancer cell lines. mRNA expression of OATP1B3, FXR, HNF1α, HNF1β, HNF3β, and GAPDH in cancer cell lines was determined by RT-PCR using specific primers (Table I) as described in Materials and Methods. An equal amount of total RNA was used for each cell line, and human liver total RNA and distilled water was included as a positive and negative control (NC), respectively. The identity of the PCR products is shown on the left.

analyzed by combined bisulfite restriction analysis (COBRA) (Fig. 2). In OATP1B3-negative cell lines (HepG2, Caco-2, and HEK293), the PCR products were sensitive to digestion with HpyCH4IV to some extent, suggesting that the genomic DNA in these cell lines was hypermethylated at −423 CpG dinucleotide. On the other hand, in OATP1B3-positive cell lines, PCR products were totally resistant to enzymatic digestion in DLD-1 and TFK-1 cells, while they were sensitive in PK-8 and PK-45P cells. These results suggest that −423 CpG dinucleotide is hypomethylated in DLD-1 and TFK-1 cells while hypermethylated in other cell lines.

DNA Methylation Profiles Around the OATP1B3 Transcriptional Start Site

There are five CpG dinucleotides in the genomic region from 800 bp upstream to 100 bp downstream around the TSS of the OATP1B3 gene. DNA methylation profiles of these CpG dinucleotides were determined by bisulfite genomic sequencing in all seven cell lines used in the present study (Fig. 3). These CpG dinucleotides were differentially methylated among cell lines. In HepG2, Caco-2, PK-8, and PK-45P cells, all the five CpG dinucleotides were almost completely hypermethylated. In HEK293 cells, the CpG dinucleotides located at −734, +70, and +73 were hypermethylated, while those at −423 and −331 were relatively hypomethylated. On the other hand, four CpG dinucleotides, located at −423, −331, +70, and +73 relative to the TSS, were significantly hypomethylated in DLD-1 and TFK-1 cells.

Activation of OATP1B3 Transcription by 5-aza-2′-Deoxycytidine Treatment

In order to elucidate the role of DNA methylation in the differential expression of OATP1B3 in cell lines, two OATP1B3-negative cell lines (HepG2 and Caco-2), and two OATP1B3-positive cell lines (DLD-1 and TFK-1) were selected and treated with 5azadC, a DNA methylation inhibitor, and then, the mRNA expression of OATP1B3 and transcription factors involved in its expression were quantified by real-time quantitative PCR. Treatment with 5azadC increased the OATP1B3 expression in HepG2 and Caco-2 cells up to 18- and 14-fold, respectively, in a concentration-dependent manner, whereas the expression was unaffected in DLD-1 and TFK-1 cells (Fig. 4a). The mRNA expression of FXR and HNF1α was about 6- and 2-fold reduced by treatment with 5azadC in HepG2 and Caco-2 cells, respectively, and that of HNF1β was about 2-fold reduced in both cell lines. There was no marked change in the mRNA expression level of HNF3β (Fig. 4b). These observations suggest that treatment with 5azadC directly affected the DNA methylation status of the genomic region encompassing the OATP1B3 promoter, leading to the transcriptional activation in OATP1B3-negative cell lines.

DISCUSSION

In the present study, the correlation between the mRNA expression and DNA methylation status of OATP1B3 was investigated in cancer cell lines. RT-PCR analysis showed that the mRNA expression of transcription factors involved in the transcriptional activity of OATP1B3 was not necessarily associated with the mRNA expression of OATP1B3 in several cancer cell lines (Fig. 1), indicating that the expression of OATP1B3 in these cancer cell lines cannot be fully accounted for by the aspect of transcription factor network. Although overexpression of HNF3β causes the reduced expression of OATP1B3 in patients with hepatocellular carcinomas (19), there was no association between the mRNA expression of OATP1B3 and HNF3β in cancer/immortalized cell lines tested. HNF3β is an essential transcription factor for visceral endoderm formation during embryonic development, and HNF3β protein has been proposed to act as a genetic potentiator of the hepatic differentiation program (27,28). It is possible that HNF3β plays a significant role in the regulation of OATP1B3 expression only in the hepatocellular carcinomas.

Bisulfite sequencing has revealed that four CpG dinucleotides located at −423, −331, +70 and +73 relative to the TSS are significantly hypomethylated in DLD-1 and TFK-1 cells compared with HepG2 and Caco-2 cells (Fig. 3). Moreover, treatment with DNA methylation inhibitor increases the

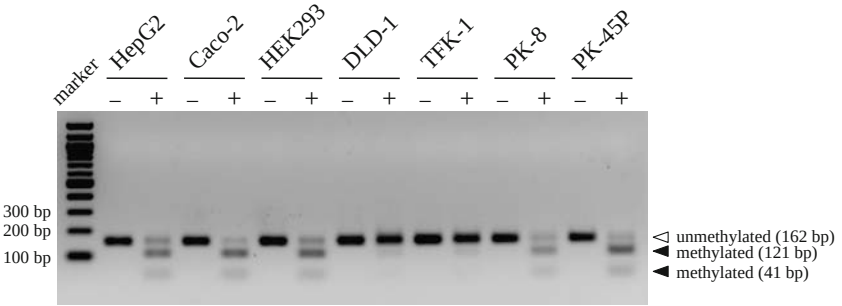

Fig. 2. Combined bisulfite restriction analysis of OATP1B3 promoter in cancer cell lines. The bisulfite PCR product from each cell line was digested with HpyCH4IV and electrophoresed using 2.0% agarose gel to evaluate the methylation status of −423 CpG dinucleotide as described in Materials and Methods. The conditions of undigested (−) and digested (+) are indicated on the top of the gel. Because only unmethylated cytosine residues are changed to thymines by the bisulfite reaction, PCR fragments from unmethylated genomic DNA are resistant to HpyCH4IV digestion, while those from methylated DNA are digested. A DNA marker was loaded on the left. The representative image of at least two independent experiments is shown.

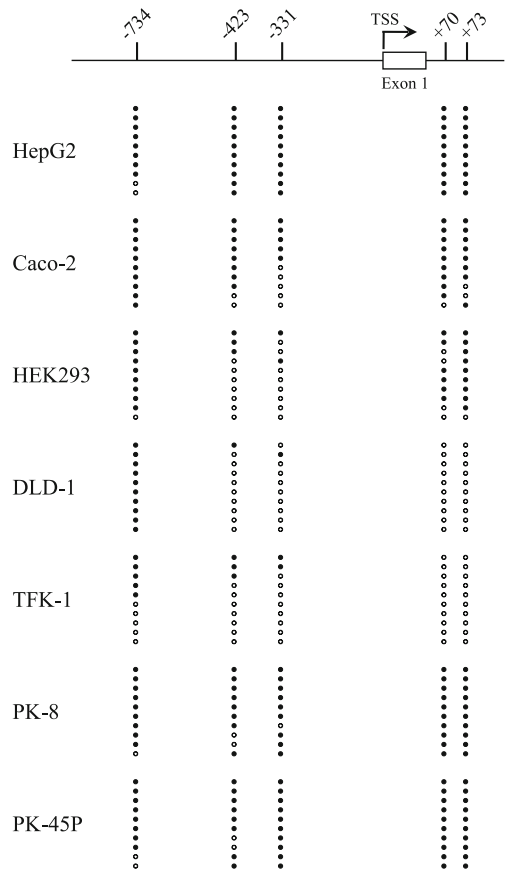

Fig. 3. DNA methylation profiles around the OATP1B3 transcriptional start site. Top, a schematic diagram of the OATP1B3 5′-flanking region. The vertical lines and numbers indicate the positions of cytosine residues of CpG dinucleotides relative to the transcriptional start site (TSS, +1). Bottom, DNA methylation status of individual CpG dinucleotides. Bisulfite sequencing analysis was performed with genomic DNAs extracted from HepG2, Caco-2, HEK293, DLD-1, TFK-1, PK-8, and PK-45P cells. Open and closed circles represent unmethylated and methylated cytosines, respectively.

mRNA expression of OATP1B3 in HepG2 and Caco-2 cells, but not in DLD-1 and TFK-1 cells (Fig. 4). In contrast, the mRNA expression of OATP1B3 transactivators, FXR, HNF1α, and HNF1β, was reduced by DNA methylation inhibitor to some extent, implying the direct interference of DNA methylation in the OATP1B3 promoter by the treatment, although the possible involvement of unknown regulatory factors cannot be ruled out. Taken together, these observations suggest the involvement of DNA methylation-dependent gene silencing in the transcriptional regulation of OATP1B3 at least in HepG2, Caco-2, DLD-1, and TFK-1 cells, where the proximal CpG dinucleotides make a larger contribution. It is generally considered that DNA hypermethylation triggers chromatin remodeling events, such as histone deacetylation and methylation, and indirectly prevents the interaction of transcription factors with their recognition sequences. The functional binding sites of FXR, HNF1α, HNF1β, and HNF3β are found within 100 bp upstream of the TSS of OATP1B3 (17–19,29), and surrounded by four CpG dinucleotides differentially methylated among cell lines. DNA hypermethylation of these four CpG dinucleotides will indirectly prevent the binding of essential transcription factors, thereby suppressing OATP1B3 transcription.

Among the OATP1B-negative cell lines, HEK293 cells exhibited a distinct DNA methylation profile, where the CpG dinucleotides located at −423 and −331 were relatively hypomethylated (Fig. 3). This may indicate that hypermethylation of other CpG dinucleotides, especially those located at +70 and +73 that are closer to the TSS, is sufficient to suppress the transcription of OATP1B3. It is worthwhile to note that unlike DLD-1 and TFK-1 cells, the genomic DNA of PK-8 and PK-45P cells, two cell lines derived from pancreatic carcinoma, is hypermethylated around the TSS of OATP1B3, while these two cell lines exhibit endogenous expression of OATP1B3 mRNA (Figs. 1, 2, and 3). There are several possibilities to account for this apparently contradictory result: 1) only the limited subpopulation of cells express OATP1B3 mRNA, which was detected by RT-PCR analysis, while bisulfite sequencing visualized the DNA methaylation status of the whole population, 2) these two cell lines utilize an alternative promoter with different TSS for the transcription of OATP1B3, and the CpG dinucleotides around that alternative TSS, if any, are significantly hypomethylated, and/or 3) positive histone marks such as acetylated H3K9 and trimethylated H3K4 are associated with OATP1B3 promoter irrespective of the densely methylated DNA. The third possibility is of particular interest: interferon

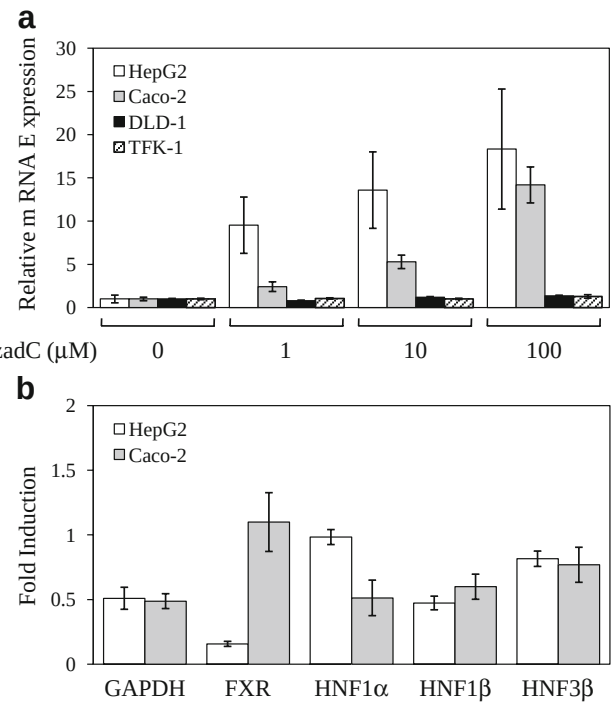

Fig. 4. Effect of 5-aza-2′-deoxycytidine treatment on the mRNA expression of OATP1B3 and transcription factors. HepG2, Caco-2, DLD-1, and TFK-1 cells were cultured for 72 h with 0, 1, 10, or 100 μM of 5azadC, and real-time quantitative PCR was performed as described in Materials and Methods. **a** The relative mRNA expression of OATP1B3 was given as a ratio with respect to that in untreated cells in each cell line. **b** Fold induction in the mRNA expression of GAPDH and transcription factors after treatment with 100 μM of 5azadC compared with untreated cells was calculated. Results are presented as the mean ± S.E. of triplicate experiments.

consensus sequence binding protein is endogenously expressed in human monoblastic U-937 cell line, where the genomic region containing the promoter region and TSS is hypermethylated but associated with active histone modifications (30). Further studies are ongoing in our group to unveil the mechanism underlying the mRNA expression of OATP1B3 in PK-8 and PK-45P cells.

A change in epigenetic signatures, including aberrant DNA methylation and histone modification, is among the most prevalent phenomena in cancer cells or tumors, and leads to drastically altered gene expression patterns (26,31,32). Aberrant methylation of the CpG islands in promoter regions of tumor-suppressor genes is involved in their transcriptional inactivation in various cancers, while genome-wide hypomethylation, mainly due to the hypomethylation of repetitive sequences, is present in most tumors, resulting in genomic instability and tumor formation. Aberrant DNA methylation has been also implicated in the alteration in the expression of a couple of drug transporters in cancer. Overexpression of P-glycoprotein/*MDR1* and Breast Cancer Resistance Protein/*ABCG2* is well known to confer chemotherapeutic resistance in malignant tumors (33). Several reports have demonstrated an inverse correlation between the gene expression of these multidrug resistance proteins and the methylation status of their promoters in human tumors or cancer cell lines (34–39). In addition to the efflux transporters, we have for the first time demonstrated the association of DNA methylation with the mRNA expression of an uptake transporter, namely OATP1B3, in at least selected cancer cell lines. Overexpression of OATP1B3 is also observed in tumor tissues of patients with non-small cell lung cancer and breast carcinoma (15,16). The fact that liver is the only normal tissue expressing OATP1B3 suggests that epigenetic signatures of OATP1B3 likely change to induce OATP1B3 expression during carcinogenesis in lung and breast tumors, although the rationale for this induction remains unknown. Future studies should determine the DNA methylation profiles of OATP1B3 gene in clinical tumor samples.

HNF1 plays a pivotal role in the transcriptional activation of liver- and kidney-specific organic anion transporters, such as OATP1B1 and OATP1B3, and OAT1, OAT3 and URAT1, respectively (18,20,21,40). We have previously demonstrated that DNA methylation in the promoter region of OAT1[1], OAT3, and URAT1 allows their kidney-specific expression (20,21). It is possible that the promoter regions of OATP1B1 and OATP1B3 are hypomethylated in the liver while hypermethylated in the kidney, thus enabling liver-specific expression. Although OATP1B1 and OATP1B3 genes are located in tandem on the same chromosome, the endogenous expression of OATP1B1 mRNA was not detected in all the cancer cell lines used in the present study and was not stimulated by 5azadC treatment (data not shown). Therefore, the regulatory mechanism of the expression of OATP1B1 is different from that of OATP1B3 in cancer cell lines. Further studies are necessary to elucidate the underlying mechanism.

In conclusion, we have revealed that CpG dinucleotides around the TSS of OATP1B3 are differentially methylated among cell lines, and the mRNA expression of OATP1B3 is stimulated by the treatment with DNA methylation inhibitor in OATP1B3-negative HepG2 and Caco-2 cells. Although there seem to be some exceptions, it is likely that the activation of OATP1B3 expression in cancer cell lines is at least partly accounted for by the relief from DNA methylation-dependent gene silencing.

[1] Saji, T., Kikuchi, R., Kusuhara, H., and Sugiyama, Y. unpublished.

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