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Epigenetic regulation of the tissue-specific expression of human UDP-glucuronosyltransferase (UGT) 1A10

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ABSTRACT

Human UDP-glucuronosyltransferase (UGT) 1A10 is not expressed in the liver; however, UGT1A10 is highly expressed in the intestine, contributing to presystemic first-pass metabolism. Earlier studies revealed that hepatocyte nuclear factor (HNF) 1α and Sp1, as well as an intestine-specific transcription factor, caudal type homeobox (Cdx) 2, are involved in the constitutive expression of UGT1A10. However, why UGT1A10 is not expressed in the liver, where HNF1α and Sp1 are abundantly expressed, is unknown. In this study, we sought to elucidate the mechanism, focusing on epigenetic regulation. Bisulfite sequence analysis revealed that the CpG-rich region (−264 to +117) around the *UGT1A10* promoter was hypermethylated (89%) in hepatocytes, whereas the *UGT1A10* promoter was hypomethylated (11%) in the epithelium of the small intestine. A luciferase assay revealed that the methylation of the *UGT1A10* promoter by *Sss*I methylase abrogated transactivity even with overexpressed Cdx2 and HNF1α. The *UGT1A10* promoter was highly methylated (86%) in liver-derived HuH-7 cells, where UGT1A10 is not expressed. In contrast, the *UGT1A10* promoter was hardly methylated (19%) in colon-derived LS180 cells, where UGT1A10 is expressed. Treatment with 5-aza-2′-deoxycitidine (5-Aza-dC), an inhibitor of DNA methylation, resulted in an increase in UGT1A10 expression only in HuH-7 cells. Moreover, overexpression of HNF1α and Cdx2 further increased UGT1A10 expression only in the presence of 5-Aza-dC. Collectively, we found that DNA hypermethylation would interfere with the binding of HNF1α and Cdx2, resulting in the defective expression of UGT1A10 in human liver. Thus, epigenetic regulation is one of the mechanisms that determine the tissue-specific expression of UGT1A10.

1. Introduction

UDP-Glucuronosyltransferases (UGTs) catalyze the glucuronidation of a variety of endogenous and exogenous compounds. In humans, there are 19 functional UGT enzymes, which are classified into three subfamilies: UGT1A, UGT2A, and UGT2B [1]. The *UGT1A* genes, which are located on chromosome 2q37, contain multiple unique first exons and common exons 2–5 and encode nine kinds of functional UGT1A enzymes [2]. The *UGT2* genes, which are located on chromosome 4q13, encode three UGT2A and seven UGT2B functional enzymes.

Human UGT enzymes are expressed in a tissue-specific manner. Most UGTs, including UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A9, UGT2B4, UGT2B10 and UGT2B7, are predominantly expressed in the liver [3,4] and expressed to a lesser extent in extra-hepatic tissues. Several UGTs are preferentially expressed in extra-hepatic tissues, including the kidney, small intestine, colon, stomach, lungs, epithelium, ovaries, testis, mammary glands and prostate. In particular, UGT1A7, UGT1A8, and UGT1A10 are highly expressed in the gastrointestinal tract, excluding the liver. This expression limits the bioavailability of orally administered drugs, such as raloxifene, naloxon, and mycophenolic acid, as well as xenobiotics, such as resveratrol and quercetin [5,6]. The intestine-specific expression of UGT1A8 and UGT1A10 was explained by transcriptional regulation through an intestine-specific transcription factor, caudal type homeobox 2 (Cdx2), as well as Sp1 and hepatocyte nuclear factor (HNF) 1α [7–10]. However, why UGT1A8 and UGT1A10 are not expressed in the liver, where Sp1 and HNF1α are abundantly expressed, remains unsolved.

The purpose of this study was to clarify the underlying mechanisms of the defective expression of UGT1A10 in the liver, focusing on epigenetic regulation. Although UGT1A8 mRNA is substantially detected in intestine, the expression of UGT1A8 protein has never been proven. In contrast, UGT1A10 protein could be clearly detected in the intestine by Western blot analysis using

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an anti-UGT1A10 specific antibody that we prepared (unpublished data). This observation is the reason we focused on UGT1A10 in this study. It is generally accepted that epigenetics, including DNA methylation and histone modification, are key regulators of tissue-dependent gene expression [11,12]. We investigated whether DNA methylation and histone modification might be determinants of the tissue-specific expression of human UGT1A10.

2. Materials and methods

2.1. Chemicals and reagents

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), goat anti-human Cdx2 polyclonal antibody (C-20), and control rabbit and goat IgGs were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Primers were commercially synthesized at the Hokkaido System Science (Sapporo, Japan). All other chemicals and solvents were of the highest grade commercially available.

2.2. Human tissues

Human liver (a 39-year-old Japanese female) and small intestine (a 49-year-old Caucasian female) were obtained from autopsy materials that were discarded after pathological investigation. The use of the human liver and small intestine was approved by the ethics committees of Kanazawa University (Kanazawa, Japan) and Iwate Medical University (Morioka, Japan).

2.3. Cell culture

Colorectal adenocarcinoma cell lines LS180, Caco-2, HT-29, and SW480 and the hepatocellular carcinoma cell line HepG2 were obtained from the American Type Culture Collection (Manassas, VA). A hepatocellular carcinoma cell lines HuH-7 were obtained from the RIKEN BioResource Center (Ibaraki, Japan). HT-29 and SW480 cells were cultured in RPMI1640 (Nissui Pharmaceutical, Tokyo, Japan) that was supplemented with 10% fetal bovine serum (Invitrogen, Carlsbad, CA). The other cells were cultured as previously described [13].

2.4. RNA isolation and real-time reverse transcription (RT)-polymerase chain reaction (PCR)

Total RNA was isolated from cell lines using RNAiso (Takara, Otsu, Japan) according to the manufacturer's protocol. The cDNA was synthesized from the total RNA using Rever Tra Ace® (Toyobo, Osaka, Japan). The UGT1A10 mRNA levels were determined by real-time RT-PCR and normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA levels as described previously [3].

2.5. Genomic DNA extraction and bisulfite reaction

Genomic DNA samples were prepared from human hepatocytes (HH268, a 54-year-old Caucasian female, Tissue Transformation Technologies), whole small intestine or epithelium of the small intestine, and cell lines using a Gentra Puregene Tissue kit (Qiagen, Valencia, CA). Five hundred nanograms of genomic DNA digested with *EcoR* I was treated with bisulfite using an EZ DNA Methylation kit (Zymo Research, Orange, CA) according to the manufacturer's protocol. The DNA fragments spanning the transcription start site (TSS) of the *UGT1A10* or *UGT1A8* genes and the 5′-flanking region of *UGT1A9* were amplified by PCR using the primer pairs that are shown in Table 1. The PCR products were cloned into the pT7Blue T-Vector (Novagen, Madison, WI). Because the primer pair for the bisulfite analysis of UGT1A8 and UGT1A10 amplifies the corresponding regions of not only UGT1A8 and UGT1A10 but also UGT1A9, clones containing UGT1A9 sequence were precluded by digestion with *Mbo* II, and clones containing UGT1A8 or UGT1A10 sequences were subjected to sequence analysis. The DNA methylation status of the sequence was analyzed using the web-based tool QUMA [14].

2.6. Construction of expression plasmids and luciferase reporter plasmids

A luciferase reporter plasmid, pCpGL-basic, which completely lacks CpG dinucleotides, was kindly provided by Dr. Rehli [15]. The 5′-flanking regions of *UGT1A9* (−955 to +29) or the promoter region of *UGT1A10* (−365 to +140), which was amplified by PCR using the human liver genome as a template, was cloned into the pCpGL-basic plasmid. The products were termed UGT1A9/pCpGL

Table 1
Sequences of oligonucleotides that were used in the present study.

Oligonucleotides	5′ to 3′ sequence	Position
Bisulfite analysis of UGT1A8 and UGT1A10		
Forward	AGAGAGTATTTGGTTGGTTAAAG	−365 to −343[a]
Reverse	ACACTACCAACAACTTCCCTACC	+118 to +140[a]
Bisulfite analysis of UGT1A9		
Forward	TTTGAAGGAGGGTATTGGAGTGATG	−754 to −730
Reverse	CCAAACCCTAAAAAACCTCTAAAATAC	−540 to −514
Cloning of promoter region of UGT1A10		
Forward	CTTTGGATCCAGAGAGTATTTGGTTGGC	−365 to −347
Reverse	CCATAGATCTGCACTACCAGCAGCTTCCC	+122 to +140
Cloning of promoter region of UGT1A9		
Forward	GGCAGCTGCAGTTGATCTTTTCCCTTTAAG	−955 to −937
Reverse	CAGAGATCTGCAGCTGAGAG	+17 to +29
ChIP assay of UGT1A10		
Forward	AATGATACTCGTGTGTTATC	−135 to −116
Reverse	AGACACACACATAAAGGAAC	+76 to +95
Cloning of Cdx2		
Forward	CCGGACCCTCGCCACCATGTA	−16 to +5
Reverse	GTGGGTCACTGGGTGACGGT	+927 to +947

Nucleotides are numbered with the TSS designated as +1 in the genomic DNA sequence of UGTs and with base A in the initiation codon ATG designated as +1 in the Cdx2 cDNA sequence. The restriction sites that were used for cloning are underlined.
[a] The numbers refer to the nucleotide position of *UGT1A10*.