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Evaluation of expression and glycosylation status of UGT1A10 in Supersomes and intestinal epithelial cells with a novel specific UGT1A10 monoclonal antibody

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Running title: Evaluation of UGT1A10 protein in human tissues

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Abbreviations: AhR, arylhydrocarbon receptor; CDX2, caudal-related homeobox protein 2 (CDX2), Endo H, Endoglycosidase H; HIM, human intestine microsomes; HKM, human kidney microsomes; HLM, human liver microsomes; HNF, hepatocyte nuclear factor; LC-MS/MS, liquid chromatography-tandem mass spectrometry; PAGE, polyacrylamide gel electrophoresis; PNGaseF, peptide:*N*-glycosidase F; UDPGA, UDP-glucuronic acid ; UGT, UDP-Glucuronosyltransferase

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Abstract

UDP-Glucuronosyltransferases (UGTs) are major phase II drug-metabolizing enzymes. Each member of the UGT family exhibits a unique but occasionally overlapping substrate specificity and tissue-specific expression pattern. Earlier studies have reported that human UGT1A10 is expressed in the gastrointestinal tract at the mRNA level, but the evaluation at the protein level, especially tissue or cellular localization, has lagged behind because of the lack of a specific antibody. In this study, we prepared a monoclonal antibody to UGT1A10 to elucidate the tissue/cellular distribution and interindividual variability of UGT1A10 protein expression. Western blot analysis revealed that the prepared antibody does not cross-react with any other human UGTs. Using this specific antibody, we observed that UGT1A10 protein is expressed in the small intestine but not in the liver or kidney. Immunohistochemical analysis revealed the expression of UGT1A10 protein in epithelial cells of the crypts and villi of the duodenum. In the small intestine microsomes from six individuals, the UGT1A10 protein levels exhibited 16-fold variability. Dopamine 3- and 4-glucuronidation, which is mainly catalyzed by UGT1A10 and by other UGT isoforms marginally, exhibited 50- to 65-fold variability, and they were not correlated with the UGT1A10 protein levels. Interestingly, the enzymatic activities of recombinant UGT1A10 in insect cells that were normalized to the UGT1A10 protein level were markedly lower than those in pooled human small intestine microsomes. Thus, the UGT1A10 antibody we generated made it possible to investigate the tissue/cellular distribution and interindividual variability of UGT1A10 protein expression for understanding the pharmacological and toxicological role of UGT1A10.

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Introduction

UDP-Glucuronosyltransferases (UGTs) constitute a superfamily of enzymes that mediate the transfer of glucuronic acid from UDP-glucuronic acid (UDPGA) to various aglycone substrates. The conjugated metabolite is more hydrophilic than the parental aglycone and is readily excreted from the body (Dutton, 1980). In humans, 19 functional UGT enzymes have been identified and are classified into three subfamilies, UGT1A, UGT2A, and UGT2B, on the basis of amino acid sequence similarity and evolutionary divergence (Mackenzie et al., 2005; Guillemette et al., 2010). The human *UGT1A* gene, located on chromosome 2q37, comprises the individual first exons and common exons 2–5 to encode nine types of UGT1A enzymes (UGT1A1 and UGT1A3 -10) (Ritter et al., 1992). UGTs are transmembrane proteins on the endoplasmic reticulum (Radominska-Pandya et al., 1999). The variable N-terminal halves of UGT1A proteins encoded from exon 1s are responsible for substrate binding, while the remaining conserved C-terminal halves contain the binding site for UDPGA (Mackenzie et al., 1997). Although the sequences of exon 1 of UGT1As are variable, whole UGT1A proteins share high degrees of sequence similarity (up to 94% amino acid similarity), by which they sometimes display overlapping substrate specificities (Nagar and Remmel, 2006; Kubota *et al*., 2007).

UGT isoforms are expressed in tissue- and cell type-dependent manners (Tukey and Strassburg, 2000; Court et al., 2012). Most of the UGT1As, including UGT1A1, 1A3, 1A4, 1A6, and 1A9, are predominantly expressed in the liver, and they are also expressed at a lower level in extrahepatic tissues (Tukey and Strassburg, 2000; Izukawa et al., 2009; Ohno and Nakajin, 2009; Court et al., 2012; Rowland et al., 2013). On the other hand, several UGTs are preferentially expressed in extrahepatic tissues, including the kidney, small intestine, colon, and stomach (Court et al., 2012). In particular, UGT1A7, UGT1A8, and UGT1A10, which belong to the highly homologous *UGT1A7-10* gene cluster, are unique in that they are mainly expressed in the gastrointestinal tract. In these tissues, genetic polymorphisms (Guillemette, 2003; Stingl et al., 2014) and induction by a variety of endogenous and exogenous compounds (Mackenzie et al., 2003) regulate inter- and intra-individual variability

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of UGT expression levels and enzyme activity, which would ultimately be responsible for the variability in the drug efficacy and toxicity as well as susceptibility to environmental chemicals.

The enzymatic activity of drug-metabolizing enzymes is assessed *in vitro* and *in vivo* using specific probe substrates for the concerned enzyme. In the case of UGTs, however, isoform-specific substrates are limited due to the overlapping substrate specificities among UGT isoforms (Oda et al., 2015). Determining the mRNA levels of each UGT isoform could be an alternative way. Although several studies have determined the absolute copy numbers of individual human UGT mRNA in tissues (Izukawa et al., 2009; Ohno and Nakajin, 2009; Court et al., 2012), they reported that the mRNA levels are not always correlated with the protein levels or enzyme activities (Izukawa et al., 2009; Oda et al., 2012; Ohtsuki et al., 2012). Compared to the mRNA levels, protein expression is generally considered to well reflect enzyme activity. Immunochemical techniques are one of the conventional and still user-friendly approaches for assessing protein levels. However, these approaches largely rely on the specificity and availability of antibodies. Currently, specific antibodies against human UGT1A1, 1A3, 1A6, 2B4, and 2B7 are commercially available, and we previously succeeded to prepare a monoclonal antibody against human UGT1A9 (Oda et al., 2012). Although an antibody against UGT1A10 is commercially available from Abcam, its specificity is not guaranteed. These facts explain why the evaluation of UGTs at the protein level by immunochemical approaches did not advance.

UGT1A10 is an extra-hepatic enzyme that has been mainly detected in the gastrointestinal tract (Strassburg et al., 1997). It catalyzes the glucuronidation of drugs such as raloxifene and SN-38 (Kemp et al., 2002; Oguri et al., 2004), and xenobiotics such as flavonoids and hydroxyl benzo[*a*]pyrenes (Zheng et al., 2002; Lewinsky et al., 2005). The expression of UGT1A10 is regulated both epigenetically and transcriptionally in response to oxidative stress or arylhydrocarbon receptor (AhR) agonists (Kalthoff et al., 2010; Oda et al., 2014). UGT1A10 is considered to play a role in the first-pass glucuronidation of some drugs, limiting the oral bioavailability. To date, its expression at the mRNA level has extensively

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been analyzed (Basu *et al*, 2004; Nakamura *et al*., 2008; Ohno and Nakajin, 2009). At the protein level, UGT1A10 levels in tissues were analyzed only by liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based quantification (Harbourt et al., 2012; Ohtsuki et al., 2012; Sato et al., 2014), but the levels determined by a specific antibody have never been reported, owing to the lack of specific antibodies to UGT1A10. Because UGT1A10 shares as high as 93 to 94% amino acid sequence similarity with UGT1A7, UGT1A8, and UGT1A9, the generation of specific antibodies to UGT1A10 is challenging. In the present study, we sought to generate a mouse monoclonal antibody that specifically recognizes UGT1A10. The prepared antibody enabled us to evaluate tissue localization and interindividual variability in the UGT1A10 expression and compare the UGT1A10 activity normalized to the UGT1A10 protein level in recombinant systems and human tissues.

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Materials and Methods

Materials

Recombinant human UGT1A1, 1A3, 1A4, 1A6, 1A7, 1A8, 1A9, and 1A10 proteins expressed in baculovirus-infected insect cells (Supersomes) were purchased from Corning (Corning, NY). Endoglycosidase H (Endo H) and peptide:*N*-glycosidase F (PNGase F) were obtained from New England Biolabs (Ipswich, MA). Precision Plus Protein Dual Color Standards was purchased from Bio-Rad (Hercules, CA). Odyssey blocking buffer and IRDye 680 LT goat anti-mouse IgG were purchased from LI-COR Biosciences (Lincoln, NE). Biotinylated goat anti-mouse IgG was obtained from Zymed (South San Francisco, CA). Normal rabbit serum and the ABC-elite kit were purchased from Vector Laboratories (Burlingame, CA). Dopamine hydrochloride was purchased from Wako Pure Chemical Industries (Osaka, Japan). Dopamine 3- and 4-glucuronides were purchased from Toronto Research Chemicals (Toronto, Canada). Alamethicin (from *Trichoderma viride*) and uridine-5'-diphosphate glucuronic acid trisodium salt (UDPGA) were obtained from Sigma-Aldrich (St. Louis, MO).

Production of anti-UGT1A10 monoclonal antibody

The peptide design, peptide synthesis, and keyhole limpet hemocyanin conjugation were carried out by Biogate (Gifu, Japan) as described in our previous study (Oda *et al*., 2012). The sequence, EDQNREFMVFAHAQWKAQAQSIFSLLMSSSSG, which corresponds to the residues 84-115 of UGT1A10, was raised as a candidate peptide. The designed peptide sequence was subjected to the protein BLAST search (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to search for homology with known protein sequences. The mouse monoclonal antibody to the peptide was produced by CLEA Japan (Tokyo, Japan) as described in our previous study (Oda *et al*., 2012).

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Tissue samples and microsome preparation

Cryopreserved human small intestine samples from six individual donors were obtained from Human and Animal Bridging Research Organization (Chiba, Japan). The donor demographics are shown in Table 1. The mucosal cells were scraped off from the intestine samples, and microsomes were prepared as described previously (Emoto et al., 2000). The protein concentrations were determined using the Bio-Rad Protein Assay kit (Bio-Rad, Hercules, CA). A pooled sample of human intestine (duodenum and jejunum) microsomes (HIMs) (three males and four females; a median age of 55 years, range: 30-70 years) and a pooled sample of human liver microsomes (HLMs) (27 males and 23 females; a median age of 52 years, range: 26-66 years) were purchased from Corning. A pooled sample of human kidney microsomes (HKMs) (three males and three females; a median age of 56 years, range: 24-60 years) was purchased from Tissue Transformation Technologies (Edison, NJ). Mouse (C57BL/6J, 6-week-old male), and rat (Sprague-Dawley, 6-week-old male) jejunum microsomes (pooled samples of five mice or three rats) were prepared as described previously (Kobayashi et al., 2012).

Cell culture and microsome preparations

The human cell lines including HepG2 (hepatocellular carcinoma), HT-29 and LS180 (colorectal adenocarcinoma), HEK293 (embryonic kidney), HK-2 (kidney tubular epithelial), and MCF-7 (breast adenocarcinoma) were purchased from American Type Culture Collection (Manassas, VA). These cells were cultured as described previously (Nakamura et al., 2008). Microsomes from these cells were prepared as described previously (Vignati et al., 2005). The protein concentrations were determined as described previously.

Western blot analysis

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For the analysis of UGT1A10, UGT Supersomes (10 or 1 μg), or microsomes from human tissues, mouse or rat jejunum, and human cell lines (30 μg) were separated by 10% SDS-polyacrylamide gel electrophoresis (PAGE). The amount of protein loaded onto gels was within the range of linearity of the signal. In some cases, recombinant UGT1A10 proteins and HIMs were treated with endoglycosidase Endo H or PNGase F. Briefly, recombinant UGT1A10 protein and HIMs were adjusted to 0.3 mg/mL and 6 mg/mL protein concentrations, respectively, in denaturing buffer containing 40 mM dithiothreitol and 0.5% SDS (in final concentrations). The samples were subsequently denatured at 95°C in a water bath for 10 min. An aliquot (1.5 μg of recombinant UGT1A10 protein or 30 μg of HIMs) was digested with 250 units of Endo H in 50 mM sodium citrate buffer (pH 5.5) or 500 units of PNGase F in 50 mM sodium phosphate buffer (pH 7.5) containing 1% Nonidet P-40 at 37°C in a water bath for 1 h, and then the samples were subjected to 10% SDS-PAGE and was electro-transferred onto Protran nitrocellulose membranes (GE Healthcare, Little Chalfont, UK). After blocking in Odyssey blocking buffer for 1 h, the membrane was incubated with 1:500-diluted anti-UGT1A10 antibody for 4 h, and then with IRDye 680 LT goat anti-mouse IgG. Infrared fluorescence signals were detected using the Odyssey Infrared Imaging system (LI-COR Biosciences).

Immunohistochemistry

A piece of small intestine (a duodenum section) was embedded in optimal cutting temperature compound (Sakura Finetek Japan, Tokyo, Japan), sectioned, and fixed with neutral formalin. After washing with water three times for 5 min each, the sections were antigen-retrieved with Liberate Antibody Binding solution (Polysciences, Warrington, PA) for 10 min, were incubated with 0.3% hydrogen peroxide in phosphate-buffered saline (PBS) for 30 min, and were blocked with 1.5% normal rabbit serum for 30 min at room temperature. The sections were subsequently incubated with 1:500-diluted anti-UGT1A10 antibody at 4°C for 16 h, incubated with biotinylated goat anti-mouse IgG at room temperature for 30 min,

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and developed with the ABC-elite kit. The sections were counterstained with Mayer's hematoxylin. As a negative control, normal mouse IgG (Santa Cruz, CA) was used instead of the anti-UGT1A10 antibody.

Dopamine glucuronidation

Dopamine 3- and 4-glucuronosyltransferase activities in UGT1A10 Supersomes, HIMs, and HLMs were measured according to a previously reported method (Itäaho et al., 2009) with modifications. A 100-µL incubation mixture contained 50 mM potassium phosphate buffer (pH 7.4), 25 µg/mL alamethicin, 5 mM $MgCl_2$, 5 mM UDPGA, 0.5 mg/mL UGT1A10 Supersomes or tissue microsomes, and 5 mM dopamine. Dopamine was dissolved in dimethylsulfoxide, and the final dimethylsulfoxide concentration in the incubation mixture was 1%. The reaction was initiated by the addition of UDPGA and incubated at 37°C in a water bath for 30 min. The reaction was terminated by the addition of 65 µL of acetonitrile. After precipitation of the protein by centrifugation at 12,000 g for 10 minutes, a 20-µL portion of the supernatant was mixed with 20 µL of acetonitrile, 100 µL of isopropanol, and 100 µL of 0.1 µM carbamazepine as an internal standard. A 5-µL portion was injected into ultra-performance LC-MS/MS (UPLC-MS/MS) for analysis.

The LC instrument used was an Acquity UPLC system consisting of a binary solvent manager, an automatic sampler, and a column oven (Waters, Milford, MA), which was equipped with a Discovery HS F5 HPLC column (2.1 mm i.d. × 150 mm, 3 µm; Supelco, Bellafonte, PA). The flow rate was set at 500 µL/min, and the column temperature was 50°C. The mobile phase was 0.1% formic acid (A) and acetonitrile (B). The gradient conditions for elution were as follows: (1) 5% of B (0-2.5 min); (2) 5-95% of B (2.5 - 3.5 min); (3) 95-5% of B (3.5 - 4.5 min); and (4) 5% of B (4.5-5 min). The LC equipment was connected to a triple quadrupole mass spectrometer (Xevo TQ MS; Waters) operated in the positive electrospray ionization mode. The working parameters for mass spectrometer were set as follows: capillary voltage, 3 kV; source temperature, 150°C; and desolvation temperature, 600°C. For the

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multiple reaction monitoring mode, the cone voltage and collision energy were set at 25 V and 20 eV, respectively, for dopamine 3- and 4-glucuronides; and 30 V and 20 eV, respectively, for carbamazepine. The analytes were detected in multiple reaction monitoring mode by monitoring the ion transitions from *m/z* 330.6 to 136.7 for dopamine 3- and 4-glucuronides, and *m/z* 236.8 to 193.9 for carbamazepine. The retention times of dopamine 4-glucuronide, dopamine 3-glucuronide, and carbamazepine were 1.45, 1.73, and 4.03 min, respectively. Quantification of dopamine 3- and 4-glucuronides was carried out by comparing the peak area ratios of authentic standards to carbamazepine as an internal standard.

Statistical analysis

Correlation analyses were performed using Pearson's correlation test. Differences between two groups were analyzed by unpaired, two-tailed student's t-test. $P < 0.05$ was considered to be statistically significant.

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Results

Specificity of the Anti-Human UGT1A10 Antibody

Because UGT families share a high degree of homology in their protein sequences, commercially available specific antibodies for each human UGT isoform are very limited. The amino acid identities of UGT1A10 with UGT1A7, UGT1A8, and UGT1A9 reached as high as 93%, 94%, and 93%, respectively (Table 2). In the present study, we prepared monoclonal antibodies by injecting mice with a peptide corresponding to amino acid residues 84-115 of UGT1A10. The amino acid homologies of the peptide of UGT1A10 (residues 84 to 115) to the corresponding residues of UGT1A7, UGT1A8, and UGT1A9 were 68, 68, and 65%, respectively. We evaluated 60 candidate antibody clones for the reactivity and specificity against recombinant human UGT1A isoforms by Western blot analysis. Among 60 clones tested, four clones specifically reacted to only UGT1A10 (data not shown). A clone with the highest reactivity among them was expanded for production of antibody. Finally, as shown in Fig. 1A, the specificity of the purified antibody was verified.

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UGT1A10 Protein Expression in Human and Rodent Tissues

Studies have previously reported the expression of UGT1A10 at the mRNA level in the human small intestine but not in the liver or kidney (Ohno and Nakajin, 2009; Court et al., 2012). We investigated whether UGT1A10 protein could be detected in human small intestine using the antibody we produced. Pooled microsomes from human small intestine (enterocytes from duodenum and jejunum sections), liver, and kidney were each analyzed by Western blot analysis. As a result, the UGT1A10 protein was detected in the small intestine but not in the liver or kidney (Fig. 1B, left), in concordance with previous reports for mRNA expression profiles. Next, we tested the reactivity of the antibody against UGTs in microsomes from mouse and rat jejunum (Fig. 1B, right). Mouse Ugt1a7c, Ugt1a9, and Ugt1a10 exhibited 75-78% amino acid sequence identities to human UGT1A10 (Table 3). In the rat, functional Ugt1a7, Ugt1a8, and Ugt1a10 proteins show 73-75% amino acid sequence identities to human

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UGT1A10, whereas the *Ugt1a9* gene is a pseudogene. As shown in the right panel of Fig. 1B, a faint band was detected in both mouse and rat jejunum microsomes, although the amino acid identities of the antigen peptide with the corresponding regions of the rodent UGTs are lower (mouse: 40-46%; rat: 40-53%) than those with the corresponding regions of the human UGT1A7-9 (65-68%) (Tables 2 and 3). The antibody might recognize rodent UGT isoforms, although the precise isoforms detected are unknown.

Reactivity of the Prepared Antibody to Glycosylated or Deglycosylated UGT1A10

N-Linked glycosylation of a protein can occur at the asparagine residue within the consensus sequence asparagine-X-threonine/serine, where X denotes any amino acid except proline (Gavel and von Heijne, 1990). *N*-Glycosylation can have an effect on protein folding, protein secretion, enzyme activity, and substrate specificity (Skropeta, 2009). As deduced from the amino acid sequence, UGT1A10 has three potential *N*-linked glycosylation sites at amino acid positions 71, 292, and 344, although there is no report for the evidence of glycosylated UGT1A10. We investigated whether UGT1A10 is glycosylated and the antibody could recognize glycosylated and de- or un-glycosylated UGT1A10. By the Western blot analysis of UGT1A10 Supersomes, three bands, which probably represent differentially glycosylated forms of UGT1A10, were observed (Fig. 1C). UGT1A10 Supersomes were treated with Endo H, which cleaves between two proximal *N*-acetylglucosamine residues of high mannose *N*-glycans. As a result, the upper and lower bands in the non-treated samples disappeared and a faster migrating band emerged (Fig. 1C, left). Notably, the band with higher mobility appeared to be darker than the sum of the upper and lower bands in the untreated UGT1A10 Supersomes. One possibility is that the de-glycosylated form of UGT1A10 was better recognized by the antibody than the glycosylated forms. The middle band in the non-treated sample may represent a glycosylated form that is relatively resistant to Endo H digestion or different post-translationally modified forms of UGT1A10. Regarding HIMs, only a band was observed. The difference in observed migration patterns of UGT1A10

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between UGT1A10 Supersomes and HIMs would be attributable to differences in the size of glycans and/or extent of glycosylation between insect and human cells. By treating HIMs with Endo H, an additional band with higher mobility, which appears to correspond to the bottom band in Endo H-treated UGT1A10 Supersomes, was observed, suggesting that UGT1A10 in HIMs was *N*-glycosylated. Next, we used PNGase F, which cleaves between the asparagine and innermost *N*-acetylglucosamine residues of high mannose, hybrid, and complex *N*-glycans. When UGT1A10 Supersomes and HIMs were treated with PNGase F, they showed the same migration patterns with those treated with Endo H (Fig. 1C, right). The middle band in the non-treated sample of UGT1A10 Supersomes was not affected by treatment with both Endo H and PNGase F, and this form of UGT1A10 was not observed in HIMs. The result indicates that the band would be a glycosidase-resistant glycosylated form or different post-translationally modified forms of UGT1A10 that is characteristic to the insect cells. Collectively, our results indicate that the prepared antibody can specifically recognize UGT1A10 irrespective of the glycosylated state.

UGT1A10 Protein Expression in Human Cell Lines

Our previous study detected UGT1A10 mRNA in human cell lines, including colorectal adenocarcinoma-derived LS180 and breast adenocarcinoma-derived MCF-7 cells (Nakamura *et al*., 2008). However, there is no report of UGT1A10 protein detection in cell lines with isoform-specific antibodies. Therefore, we tested whether UGT1A10 protein is detectable in human cell lines using microsomes from six types of human tissue-derived cell lines, HepG2, HT-29, LS180, HEK293, HK-2, and MCF-7. As a result, no band was detected by the antibody we generated, indicating that the endogenous UGT1A10 protein levels in these cells are not high enough to be detectable by Western blot analysis (Fig. 1D).

Immunohistochemical Analysis of UGT1A10 in Human Gastrointestinal Tissues

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After the confirmation of its specificity, we performed immunohistochemical analysis using the anti-UGT1A10 antibody to examine the localization of UGT1A10 protein in human duodenum tissues (Fig. 2). It was demonstrated that UGT1A10 protein was almost exclusively detected in the epithelial cells of the villi (Fig. 2, region A) and crypts (region B), but not in the muscularis externa (region C). These results indicated that our prepared antibody could be used for immunohistochemical study to assess UGT1A10 protein localization.

Expression Levels of UGT1A10 Protein in Individual Human Intestines

We evaluated the interindividual variability of the UGT1A10 protein levels in six HIMs and found a 16-fold difference (Fig. 3A, D and E). Because dopamine was reported to be a relatively specific substrate for UGT1A10 (Itäaho et al., 2009), although other UGT isoforms also metabolize it to a minor extent, we measured dopamine glucuronidation in HIM as indices of UGT1A10 activity. In six individual HIMs, dopamine 3- and 4-glucuronosyltransferase activities ranged from 0.54 ± 0.11 to 34.95 ± 4.18 and from 2.22 ± 0.40 to 112.05 ± 11.15 pmol/min/mg protein, respectively (Fig. 3B). A significant positive correlation (r = 0.99, p < 0.0001) was observed between the two activities. The dopamine 3- (r = 0.33, p = 0.35, Fig. 3D) and 4-glucuronosyltransferase activities (r = 0.31, p = 0.50, Fig. 3E) were not correlated with the UGT1A10 protein levels. Next, we determined dopamine glucuronidation in pooled HLMs and HIMs and UGT1A10 Supersomes (Fig. 3B). Unexpectedly, dopamine 3- and 4-glucuronsyltransferase activities were detected in pooled HLMs with 36.74 ± 1.32 and 104.49 ± 9.26 pmol/min/mg, respectively, which were approximately half of those in pooled HIMs (64.54 ± 11.56 and 247.43 ± 6.92 pmol/min/mg). These results suggest that UGT isoforms other than UGT1A10 would contribute to the activities in the liver because UGT1A10 is not expressed in the liver. Interestingly, although the expression level of UGT1A10 protein in Supersomes (100 ± 14 unit of UGT1A10 protein/μg protein) was apparently higher than that in pooled HIMs (1.8 ± 0.3 unit of

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UGT1A10 protein/μg protein) (Fig. 3A), dopamine 3- and 4-glucuronsyltransferase activities in the former source (0.82 ± 0.17 and 1.81 ± 0.15 pmol/min/mg) were much lower than those in pooled HIMs (64.54 ± 11.56 and 247.43 ± 6.92 pmol/min/mg). Accordingly, dopamine 3- and 4-glucuronosyltransferase activities normalized to the UGT1A10 protein level were calculated to be 0.008 ± 0.001 and 0.018 ± 0.001 fmol/min/unit in UGT1A10 Supersomes and 35.9 ± 4.4 and 138.5 ± 30.0 fmol/min/unit in pooled HIMs, respectively (Fig. 3C). These results suggest that UGT1A10 Supersomes include a considerable amount of the inactive form.

Discussion

UGT1A10 is well-documented to be a pharmacologically and physiologically important UGT isoform because it metabolizes various xenobiotics in the intestine to limit their intestinal absorption (Cheng et al., 1999). A previous study has reported that UGT1A10 mRNA is expressed in the small intestine and colon (Court et al., 2012). Furthermore, absolute UGT1A10 protein level in the small intestine was also reported using LC-MS/MS-based approaches (Harbourt et al., 2012; Sato et al., 2014). However, further information, such as tissue localization and post-translational modification of UGT1A10 protein, is still lacking due to the unavailability of a specific antibody against UGT1A10. In this study, we successfully produced a mouse monoclonal antibody and evaluated tissue localization and interindividual variability of UGT1A10 protein expression in the human small intestine.

By Western blot analysis employing the generated antibody, UGT1A10 protein was detected in the small intestine (duodenum and jejunum) but not in the liver and kidney (Fig. 1B). Immunohistochemical analyses revealed that UGT1A10 protein was localized in the villi and crypts of the epithelium but not in the submucosal region (Fig. 2). Our findings are in agreement with previous observations as follows: 1) UGT1A protein was detected in the epithelial layer and crypts in ileum tissue by immunohistochemistry using an anti-UGT1A

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antibody that recognizes all UGT1A isoforms (Strassburg et al., 2000); 2) UGT1A10 mRNA was detected in duodenal and ileal mucosa by *in situ* hybridization analysis (Basu et al., 2004); 3) the absolute copy number of UGT1A10 mRNA was shown to be high in the small intestine and colon by quantitative real-time RT-PCR (Ohno and Nakajin, 2009; Court et al., 2012); 4) UGT1A10 protein was detected in the small intestine by LC-MS/MS peptide quantification (Harbourt *et al*., 2012; Sato *et al*., 2014). Hence, our detection of UGT1A10 protein in the human small intestine confirms the observations in earlier studies, supporting the specificity of the generated antibody. The intestinal epithelium is composed of luminal protrusions and invaginations into the submucosa, known as villi and crypts of Lieberkühn, respectively (Sancho et al., 2004). The crypt harbors proliferative undifferentiated stem cells and mucus-secreting Paneth cells, and the stem cells differentiate to enterocytes, enteroendocrine cells, and goblet cells at the villi. It has been reported that the glucuronosyltransferase activity of 3-hydroxybenzo[*a*]pyrene, a substrate for human UGT1A10 (Dellinger *et al*., 2006), was detected in both the villi and crypts, and the activity in the villous tip was significantly prominent and gradually declined toward the crypt in the rat intestine (Dubey and Singh, 1988). Although species differences may exist, the highly proliferative crypt stem cells may adapt to environmental exposure, enabling the environmental chemicals to be eliminated quickly through glucuronidation and offering protection to these highly proliferative cells against toxic xenobiotics.

We measured dopamine glucuronidation as indices of UGT1A10 activity because dopamine has been reported to be mainly metabolized by UGT1A10 (Itäaho et al., 2009). The dopamine glucuronosyltransferase activities were substantially detected in the human liver where UGT1A10 is absent, suggesting that the activity would be catalyzed by other UGT isoforms. Itäaho *et al*. (2009) reported that UGT1A1, 1A3, 1A6, 1A7, 1A8, 1A9, 2A1, 2A3, 2B7, 2B11, 2B15, and 2B17 showed marginal dopamine glucuronidation. Therefore, the activities in HLMs would be due to UGT1A1, 1A3, 1A6, 2A1, 2A3, 2B7, 2B15, or 2B17, which are expressed in the human liver. Because UGT1A1, 1A3, 1A6, 1A7, 1A8, 1A9, and 2B7 are expressed in the small intestine (Court *et al*., 2012), we cannot ignore that they may

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also contribute to the dopamine glucuronidation in HIMs. This might be one of the reasons for the finding that dopamine glucuronosyltransferase activities normalized to the UGT1A10 protein level in Supersomes were considerably lower than those in HIMs (Fig. 3C) and that the dopamine 3- and 4-glucuronosyltransferase activities were not correlated with the UGT1A10 protein levels (Figs. 3D and 3E). However, we believe, even with the above reason, that it would be true that UGT1A10 Supersomes have low specific activity because we had found a similar phenomenon for UGT1A9 (Oda *et al*., 2012). In addition, Troberg *et al.* (in press) recently reported that UGT1A10 Supersomes showed considerably lower activities than in-house prepared histidine-tagged UG1A10 expressed in insect cells using morphine, estrone, 7-methyl-4-aminocoumarin, and bavachinin as substrates. These results suggest that the evaluation of glucuronidation using UGT1A10 Supersomes would result in the underestimation of the contribution of UGT1A10.

Another possible reason for the lower specific activity of UGT1A10 Supersomes than that in the human small intestine might be due to the difference in post-translational modification. In this study, we showed that the migration patterns of UGT1A10, as well as the susceptibility to Endo H or PNGase F, were different between HIMs and the recombinant system (Fig. 2C). It has been reported that glucuronsyltransferase activity is modulated by the *N*-glycosylation (Barbier et al., 2000; Nakajima et al., 2010) and phosphorylation (Basu et al., 2005) of UGT proteins. Thus, the difference in the post-translational modifications of UGT1A10 proteins between enzyme sources would contribute to the difference in the specific activity. Additionally, the formation of dimers or oligomers of UGT may also be involved. It has been demonstrated that UGT1A10 forms homo-oligomers or hetero-oligomers with UGT1A1 in COS cells (Operana and Tukey, 2007). We and other research groups have reported that the enzymatic activities of some UGT isoforms were modulated with other UGT isoforms by hetero-oligomerization (Fujiwara et al., 2007a; Fujiwara et al., 2007b; Fujiwara et al., 2010). Because, in a recombinant system, a single UGT isoform is expressed, hetero-oligomerization is physically impossible. Thus, the absence of hetero-oligomerization

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might be an additional reason for the low activity normalized to the UGT protein level in the recombinant system.

We observed a moderate (16-fold) interindividual variability in the intestinal UGT1A10 protein levels, although the sample number was limited (Fig. 3A). Some previous studies have investigated the underlying mechanisms for the variability of UGT1A10 expression, focusing on genetic polymorphisms, transcription factors, and epigenetic modifications. Currently, three missense polymorphisms of *UGT1A10* have been identified, being designated *UGT1A10*2*, containing the single-nucleotide polymorphism (SNP) 415G>A leading to an amino acid change Glu139Lys, *UGT1A10*3* containing SNP 719C>T leading to Thr240Met, and *UGT1A10*4* containing SNP 730C>A leading to Leu244Ile (Elahi et al., 2003). In particular, the *UGT1A10*2* allelic variant showed low UGT1A10 mRNA expression in the lung tissue and results in low enzyme activity in the recombinant system (Dellinger et al., 2006). In addition, a SNP -1271 C>G and a 1664-bp deletion between nucleotides -190 to -1856 were identified (Balliet et al., 2010). It was reported that reporter constructs with the 1664-bp deletion resulted in an increase in luciferase activity compared to the wild-type promoter constructs. Such polymorphisms could be contributing factors for variability in UGT1A10 expression. Regarding transcription factors and epigenetic modifications, an intestinal transcription factor caudal-related homeobox protein 2 (CDX2), hepatocyte nuclear factor (HNF) 1α (Gregory et al., 2004), aryl hydrocarbon receptor (AhR), and nuclear factor erythroid 2-related factor 2 (Kalthoff et al., 2010) have been shown to activate UGT1A10 transcription. Especially, the induction of UGT1A10 by CDX2 and HNF1α is known to be synergistic (Gregory et al., 2004) and is elicited by DNA hypomethylation of the proximal promoter in the small intestine (Oda et al., 2014). Thus, the expression levels of transcription factors, DNA methylation status, AhR ligand exposure, and oxidative stress would affect the variability in UGT1A10 expression. Furthermore, a recent study demonstrated that microRNA-491-3p represses UGT1A1 protein expression through base pairing to the 3′-untranslated region that is common to all UGT1A isoforms (Dluzen et al., 2014). Therefore, it is possible that the expression of UGT1A10 is also regulated by miR-491-3p. Taken

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together, the abovementioned factors may be responsible for the interindividual variability of UGT1A10 expression.

Generally, the enzymatic activity of a given enzyme is correlated with protein expression rather than with mRNA expression, as actually demonstrated for UGT1A9 (Oda et al., 2012). Therefore, the protein expression is recognized as a more proper parameter for the functional activity of UGTs. However, the insufficient availability of isoform-specific antibodies has limited quantitative evaluation of UGT isoforms at the protein level. As an alternative approach, some researchers have recently established LC-MS/MS-based absolute protein quantification (Harbourt et al., 2012; Ohtsuki et al., 2012). This method is useful because it can provide absolute expression level of any protein if an appropriate standard peptide was used; however, versatility is low because it requires expensive instrumentation. In contrast, immunochemical analysis is simpler and less costly, once specific antibodies are prepared. As for the detection of UGT1A10 in the human kidney, conflicting data are shown. In previous studies, UGT1A10 mRNA was not detected (Court *et al.,* 2012) and UGT1A10 protein was not detected by LC-MS/MS analysis (Sato *et al*., 2014). The present study supported the absence of UGT1A10 protein in the kidney by Western blot analysis. In contrast, an earlier study by Harbourt *et al.* (2012) demonstrated the detection of UGT1A10 protein in the kidney by LC-MS/MS analysis. Likely explanations for the discrepancy between two results by LC-MS/MS analysis may include differences in the samples used, the specificity of the peptide monitored in the LC-MS/MS analysis, and sensitivity of the used instrument.

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Conclusions

We produced a monoclonal antibody that specifically recognizes human UGT1A10. By Western blot analysis, it was demonstrated that human UGT1A10 protein is substantially expressed in the small intestine but not in the liver and kidney. Furthermore, immunohistochemical analysis showed that UGT1A10 is localized in the epithelial cells of

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the villi and crypts of gastrointestinal tissues. Interestingly, it was demonstrated that enzymatic activities normalized to UGT1A10 protein were extremely lower in a commercially available recombinant system than in the human small intestine. With increased research interest in UGT1A10, this antibody would be useful to study the clinical significance of UGT1A10.

Conflicts of interest

The authors have no conflicts of interest to declare.

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Authorship Contributions

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

Conducted experiments: Oda, Kato, Hatakeyama, and Iwamura.

Contributed new reagents or analytic tools: Hatakeyama.

Performed data analysis: Oda, Kato, and Nakajima.

Wrote or contributed to the writing of the manuscript: Oda, Kato, Kume, and Nakajima.

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References

Balliet RM, Chen G, Dellinger RW, and Lazarus P (2010) UDP-glucuronosyltransferase 1A10: activity against the tobacco-specific nitrosamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol, and a potential role for a novel UGT1A10 promoter deletion polymorphism in cancer susceptibility. *Drug Metab Dispos* **38:**484-490.

Barbier O, Girard C, Breton R, Belanger A, and Hum DW (2000) N-glycosylation and residue 96 are involved in the functional properties of UDP-glucuronosyltransferase enzymes. *Biochemistry* **39:**11540-11552.

Basu NK, Kole L, Kubota S, and Owens IS (2004) Human UDP-glucuronosyltransferases show atypical metabolism of mycophenolic acid and inhibition by curcumin. *Drug Metab Dispos* **32:**768-773.

Basu NK, Kovarova M, Garza A, Kubota S, Saha T, Mitra PS, Banerjee R, Rivera J, and Owens IS (2005) Phosphorylation of a UDP-glucuronosyltransferase regulates substrate specificity. *Proc Natl Acad Sci U S A* **102:**6285-6290.

Cheng Z, Radominska-Pandya A, and Tephly T (1999) Studies on the substrate specificity of human intestinal UDP-glucuronosyltransferases 1A8 and 1A10. *Drug Metab Dispos* **27:**1165-1170.

Court MH, Zhang X, Ding X, Yee KK, Hesse LM, and Finel M (2012) Quantitative distribution of mRNAs encoding the 19 human UDP-glucuronosyltransferase enzymes in 26 adult and 3 fetal tissues. *Xenobiotica* **42:**266-277.

Dellinger RW, Fang JL, Chen G, Weinberg R, and Lazarus P (2006) Importance of UDP-glucuronosyltransferase 1A10 (UGT1A10) in the detoxification of polycyclic aromatic hydrocarbons: decreased glucuronidative activity of the UGT1A10^{139Lys} isoform. *Drug Metab Dispos* **34:**943-949.

Dluzen DF, Sun D, Salzberg AC, Jones N, Bushey RT, Robertson GP, and Lazarus P (2014) Regulation of UDP-glucuronosyltransferase 1A1 expression and activity by microRNA 491-3p. *J Pharmacol Exp Ther* **348:**465-477.

Dubey RK and Singh J (1988) Localization and characterization of drug-metabolizing enzymes along the villus-crypt surface of the rat small intestine--II. Conjugases. *Biochem Pharmacol* **37:**177-184.

Dutton GJ (1980) Acceptor substrates of UDP glucuronosyltransferase and their assay, in *Glucuronidation of Drugs and Other Compounds* (Dutton GJ ed) pp 69-78, CRC Press, Boca Raton, FL.

Elahi A, Bendaly J, Zheng Z, Muscat JE, Richie JP, Jr., Schantz SP, and Lazarus P (2003) Detection of UGT1A10 polymorphisms and their association with orolaryngeal carcinoma risk. *Cancer* **98:**872-880.

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DMD Fast Forward. Published on July 11, 2017 as DOI: 10.1124/dmd.117.075291
This article has not been copyedited and formatted. The final version may differ from this version.

Emoto C, Yamazaki H, Yamasaki S, Shimada N, Nakajima M, and Yokoi T (2000) Characterization of cytochrome P450 enzymes involved in drug oxidations in mouse intestinal microsomes. *Xenobiotica* **30:**943-953.

Fujiwara R, Nakajima M, Oda S, Yamanaka H, Ikushiro S, Sakaki T, and Yokoi T (2010) Interactions between human UDP-glucuronosyltransferase (UGT) 2B7 and UGT1A enzymes. *J Pharm Sci* **99:**442-454.

Fujiwara R, Nakajima M, Yamanaka H, Katoh M, and Yokoi T (2007a) Interactions between human UGT1A1, UGT1A4, and UGT1A6 affect their enzymatic activities. *Drug Metab Dispos* **35:**1781-1787.

Fujiwara R, Nakajima M, Yamanaka H, Nakamura A, Katoh M, Ikushiro S, Sakaki T, and Yokoi T (2007b) Effects of coexpression of UGT1A9 on enzymatic activities of human UGT1A isoforms. *Drug Metab Dispos* **35:**747-757.

Gavel Y and von Heijne G (1990) Sequence differences between glycosylated and non-glycosylated Asn-X-Thr/Ser acceptor sites: implications for protein engineering. *Protein Eng* **3:**433-442.

Gregory PA, Lewinsky RH, Gardner-Stephen DA, and Mackenzie PI (2004) Regulation of UDP glucuronosyltransferases in the gastrointestinal tract. *Toxicol Appl Pharmacol* **199:**354-363.

Guillemette C (2003) Pharmacogenomics of human UDP-glucuronosyltransferase enzymes. *Pharmacogenomics J* **3:**136-158.

Guillemette C, Levesque E, Harvey M, Bellemare J, and Menard V (2010) UGT genomic diversity: beyond gene duplication. *Drug Metab Rev* **42:**24-44.

Harbourt DE, Fallon JK, Ito S, Baba T, Ritter JK, Glish GL, and Smith PC (2012) Quantification of human uridine-diphosphate glucuronosyl transferase 1A isoforms in liver, intestine, and kidney using nanobore liquid chromatography-tandem mass spectrometry. *Anal Chem* **84:**98-105.

Itäaho K, Court MH, Uutela P, Kostiainen R, Radominska-Pandya A, and Finel M (2009) Dopamine is a low-affinity and high-specificity substrate for the human UDP-glucuronosyltransferase 1A10. *Drug Metab Dispos* **37:**768-775.

Izukawa T, Nakajima M, Fujiwara R, Yamanaka H, Fukami T, Takamiya M, Aoki Y, Ikushiro S, Sakaki T, and Yokoi T (2009) Quantitative analysis of UDP-glucuronosyltransferase (UGT) 1A and UGT2B expression levels in human livers. *Drug Metab Dispos* **37:**1759-1768.

Kalthoff S, Ehmer U, Freiberg N, Manns MP, and Strassburg CP (2010) Interaction between oxidative stress sensor Nrf2 and xenobiotic-activated aryl hydrocarbon receptor in the regulation of the human phase II detoxifying UDP-glucuronosyltransferase 1A10. *J Biol Chem* **285:**5993-6002.

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DMD Fast Forward. Published on July 11, 2017 as DOI: 10.1124/dmd.117.075291
This article has not been copyedited and formatted. The final version may differ from this version.

Kemp DC, Fan PW, and Stevens JC (2002) Characterization of raloxifene glucuronidation in vitro: contribution of intestinal metabolism to presystemic clearance. *Drug Metab Dispos* **30:**694-700.

Kobayashi Y, Fukami T, Nakajima A, Watanabe A, Nakajima M, and Yokoi T (2012) Species differences in tissue distribution and enzyme activities of arylacetamide deacetylase in human, rat, and mouse. *Drug Metab Dispos* **40:**671-679.

Kubota T, Lewis BC, Elliot DJ, Mackenzie PI, and Miners JO (2007) Critical roles of residues 36 and 40 in the phenol and tertiary amine aglycone substrate selectivities of UDP-glucuronosyltransferases 1A3 and 1A4. *Mol Pharmacol* **72:** 1054-1062.

Kurkela M, Patana AS, Mackenzie PI, Court MH, Tate CG, Hirvonen J, Goldman A, and Finel M (2007) Interactions with other human UDP-glucuronosyltransferases attenuate the consequences of the Y485D mutation on the activity and substrate affinity of UGT1A6. *Pharmacogenet Genomics* **17:**115-126.

Lewinsky RH, Smith PA, and Mackenzie PI (2005) Glucuronidation of bioflavonoids by human UGT1A10: structure-function relationships. *Xenobiotica* **35:**117-129.

Mackenzie PI, Bock KW, Burchell B, Guillemette C, Ikushiro S, Iyanagi T, Miners JO, Owens IS, and Nebert DW (2005) Nomenclature update for the mammalian UDP glycosyltransferase (*UGT*) gene superfamily. *Pharmacogenet Genomics* **15:**677-685.

Mackenzie PI, Gregory PA, Gardner-Stephen DA, Lewinsky RH, Jorgensen BR, Nishiyama T, Xie W, and Radominska-Pandya A (2003) Regulation of UDP glucuronosyltransferase genes. *Curr Drug Metab* **4:**249-257.

Mackenzie PI, Owens IS, Burchell B, Bock KW, Bairoch A, Belanger A, Fournel-Gigleux S, Green M, Hum DW, Iyanagi T, Lancet D, Louisot P, Magdalou J, Chowdhury JR, Ritter JK, Schachter H, Tephly TR, Tipton KF, and Nebert DW (1997) The UDP glycosyltransferase gene superfamily: recommended nomenclature update based on evolutionary divergence. *Pharmacogenetics* **7:**255-269.

Nagar S and Remmel RP (2006) Uridine diphosphoglucuronosyltransferase pharmacogenetics and cancer. *Oncogene* **25:**1659-1672.

Nakajima M, Koga T, Sakai H, Yamanaka H, Fujiwara R, and Yokoi T (2010) *N*-Glycosylation plays a role in protein folding of human UGT1A9. *Biochem Pharmacol* **79:**1165-1172.

Nakamura A, Nakajima M, Yamanaka H, Fujiwara R, and Yokoi T (2008) Expression of UGT1A and UGT2B mRNA in human normal tissues and various cell lines. *Drug Metab Dispos* **36:**1461-1464.

Oda S, Fukami T, Yokoi T, and Nakajima M (2014) Epigenetic regulation of the tissue-specific expression of human UDP-glucuronosyltransferase (UGT) 1A10. *Biochem Pharmacol* **87:**660-667.

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DMD Fast Forward. Published on July 11, 2017 as DOI: 10.1124/dmd.117.075291
This article has not been copyedited and formatted. The final version may differ from this version.

Oda S, Fukami T, Yokoi T, and Nakajima M (2015) A comprehensive review of UDP-glucuronosyltransferase and esterases for drug development. *Drug Metab Pharmacokinet* **30:**30-51.

Oda S, Nakajima M, Hatakeyama M, Fukami T, and Yokoi T (2012) Preparation of a specific monoclonal antibody against human UDP-glucuronosyltransferase (UGT) 1A9 and evaluation of UGT1A9 protein levels in human tissues. *Drug Metab Dispos* **40:**1620-1627.

Oguri T, Takahashi T, Miyazaki M, Isobe T, Kohno N, Mackenzie PI, and Fujiwara Y (2004) UGT1A10 is responsible for SN-38 glucuronidation and its expression in human lung cancers. *Anticancer Res* **24:**2893-2896.

Ohno S and Nakajin S (2009) Determination of mRNA expression of human UDP-glucuronosyltransferases and application for localization in various human tissues by real-time reverse transcriptase-polymerase chain reaction. *Drug Metab Dispos* **37:**32-40.

Ohtsuki S, Schaefer O, Kawakami H, Inoue T, Liehner S, Saito A, Ishiguro N, Kishimoto W, Ludwig-Schwellinger E, Ebner T, and Terasaki T (2012) Simultaneous absolute protein quantification of transporters, cytochromes P450, and UDP-glucuronosyltransferases as a novel approach for the characterization of individual human liver: comparison with mRNA levels and activities. *Drug Metab Dispos* **40:**83-92.

Operana TN and Tukey RH (2007) Oligomerization of the UDP-glucuronosyltransferase 1A proteins: homo- and heterodimerization analysis by fluorescence resonance energy transfer and co-immunoprecipitation. *J Biol Chem* **282:**4821-4829.

Radominska-Pandya A, Czernik PJ, Little JM, Battaglia E, and Mackenzie PI (1999) Structural and functional studies of UDP-glucuronosyltransferases. *Drug Metab Rev* **31:**817-899.

Ritter JK, Yeatman MT, Ferreira P, and Owens IS (1992) Identification of a genetic alteration in the code for bilirubin UDP-glucuronosyltransferase in the *UGT1* gene complex of a Crigler-Najjar type I patient. *J Clin Invest* **90:**150-155.

Rowland A, Miners JO, and Mackenzie PI (2013) The UDP-glucuronosyltransferases: their role in drug metabolism and detoxification. *Int J Biochem Cell Biol* **45:**1121-1132.

Sancho E, Batlle E, and Clevers H (2004) Signaling pathways in intestinal development and cancer. *Annu Rev Cell Dev Biol* **20:**695-723.

Sato Y, Nagata M, Tetsuka K, Tamura K, Miyashita A, Kawamura A, and Usui T (2014) Optimized methods for targeted peptide-based quantification of human uridine 5'-diphosphate-glucuronosyltransferases in biological specimens using liquid chromatography-tandem mass spectrometry. *Drug Metab Dispos* **42:**885-889.

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DMD Fast Forward. Published on July 11, 2017 as DOI: 10.1124/dmd.117.075291
This article has not been copyedited and formatted. The final version may differ from this version.

Skropeta D (2009) The effect of individual N-glycans on enzyme activity. *Bioorg Med Chem* **17:**2645-2653.

Stingl JC, Bartels H, Viviani R, Lehmann ML, and Brockmoller J (2014) Relevance of UDP-glucuronosyltransferase polymorphisms for drug dosing: A quantitative systematic review. *Pharmacol Ther* **141:**92-116.

Strassburg CP, Kneip S, Topp J, Obermayer-Straub P, Barut A, Tukey RH, and Manns MP (2000) Polymorphic gene regulation and interindividual variation of UDP-glucuronosyltransferase activity in human small intestine. *J Biol Chem* **275:**36164-36171.

Strassburg CP, Oldhafer K, Manns MP, and Tukey RH (1997) Differential expression of the UGT1A locus in human liver, biliary, and gastric tissue: identification of *UGT1A7* and *UGT1A10* transcripts in extrahepatic tissue. *Mol Pharmacol* **52:**212-220.

Troberg J, Jarvinen E, Ge GB, Yang L, and Finel M (2016) UGT1A10 Is a High Activity and Important Extrahepatic Enzyme: Why Has Its Role in Intestinal Glucuronidation Been Frequently Underestimated? *Mol Pharm*, in press.

Tukey RH and Strassburg CP (2000) Human UDP-glucuronosyltransferases: metabolism, expression, and disease. *Annu Rev Pharmacol Toxicol* **40:**581-616.

Vignati L, Turlizzi E, Monaci S, Grossi P, Kanter R, and Monshouwer M (2005) An in vitro approach to detect metabolite toxicity due to CYP3A4-dependent bioactivation of xenobiotics. *Toxicology* **216:**154-167.

Zheng Z, Fang JL, and Lazarus P (2002) Glucuronidation: an important mechanism for detoxification of benzo[*a*]pyrene metabolites in aerodigestive tract tissues. *Drug Metab Dispos* **30:**397-403.

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Figure Legends

Figure 1. Western blot analyses using the monoclonal antibody to human UGT1A10. (A) Recombinant UGT1As Supersomes (10 μg) and (B) pooled microsomes from human small intestine, liver, and kidney (30 μg) (left panel) as well as pooled microsomes from human small intestine and mouse and rat jejunum (30 μg) (right panel) were subjected to Western blot analysis using the UGT1A10 antibody. (C) Endo H (left panel) or PNGase F (right panel)-treated (+) and non-treated (-) UGT1A10 Supersomes (1.5 μg) and HIMs (30 μg) were analyzed by Western blotting using the UGT1A10 antibody. Arrowheads represent UGT1A10. (D) Pooled HIMs and microsomes from human cell lines (30 μg) were analyzed by Western blotting using the UGT1A10 antibody. HIMs, human small intestine microsomes; HLMs, human liver microsomes; HKMs, human kidney microsomes; MJMs, mouse jejunum microsomes; RJMs, rat jejunum microsomes; M, pre-stained marker (Precision Plus Protein Dual Color molecular weight standards).

Figure 2. Immunohistochemical analysis of UGT1A10 in human duodenum tissues. Immunohistochemical staining was performed in human duodenum tissues using the anti-UGT1A10 antibody. Original magnification, ×40 (leftmost image); other images, ×200 magnification of the regions A, B, and C. A, mucosal epithelia; B, submucosa; C, muscularis externa. Strong immunostaining was observed in the epithelial cells of the crypts and villi in the duodenum.

Figure 3. Interindividual variability of the UGT1A10 protein levels and dopamine 3- and 4-glucronosyltransferase activities in human small intestine microsomes. (A) Six-individual donors-derived and pooled HIMs (30 μg), as well as UGT1A10 Supersomes (1 μg), were subjected to Western blot analysis using the UGT1A10 antibody. (B) Dopamine 3- and

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4-glucronosyltransferase activities were determined at 5 mM dopamine as a substrate in 6 individual donors and pooled HIMs, pooled HLMs, and UGT1A10 Supersomes. (C) Dopamine 3- and 4-glucuronosyltransferase activities in pooled HIMs and UGT1A10 Supersomes were normalized to UGT1A10 protein levels. The enzyme activities are expressed as femtomoles per minute per unit of UGT1A10 protein. (D and E) The correlations between the UGT1A10 protein levels and dopamine 3- or 4-glucuronosyltransferase activities in 6 individual donors and pooled HIMs were analyzed. The UGT1A10 protein levels are expressed as the levels relative to that of the lowest sample. The data were represented means ± SD or means of triplicate determinations. ***, $p < 0.001$. NS, not significant.

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Table 1. Information of intestinal tissue donors

No.	Experiment Performed	Sex	Age (yr)	Race	Cause of Death
1	WB	F	17	Caucasian	Blunt head injury
2	WB	F	54	Caucasian	Intracranial hemorrhage
3	WB	F	49	Caucasian	Intracranial hemorrhage
4	WB	M	69	Caucasian	Cardiac arrest/Abdominal aortic aneurysm
5	WB, IHC	M	57	Caucasian	Intracranial bleed
6	WB	M	60	Caucasian	Cerebrovascular accident (Stroke)

WB, Western blotting; IHC, immunohistochemistry; M, male; F, female.

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Table 2. Sequence alignment of the antigen peptide of human UGT1A10 with the other UGT1As

Isoform	Accession No.	Sequence	Identity with UGT1A10 (%)	
			84-115	Full-length
UGT1A10	AAB81537	$_{84}$---EDQNREFMVFAHAQWKAQAQSIFSLLMSSSSG-$_{115}$		
UGT1A1	P22309	$_{84}$-QR**ED**VKES**F**VSLG**H**NVFEN--D**S**FLQRVIKTYKK-$_{115}$	12	65
UGT1A3	P35503	$_{84}$WTQDEFD**R**HVLGHTQLYFET--EHFLKKFFR**S**MA--$_{115}$	12	66
UGT1A4	P22310	$_{84}$WTQKEFD**R**VTLGYTQGFFET--EHLLKRYSR**S**MA--$_{115}$	6	65
UGT1A5	P35504	$_{84}$WTQDEFD**R**LLLGHTQSFFET--EHLLMKFSRRMA--$_{115}$	6	66
UGT1A6	P19224	$_{84}$--Q**E**ELKN**R**YQS**F**GNNHFAE--R**S**FLTAPQTEYRNN$_{115}$	6	67
UGT1A7	NP_061950	$_{84}$---**EDQ**D**REFMVFA**D**A**R**W**T**A**PLR**S**A**FSLL**T**SSS**N**G**-$_{115}$	68	93
UGT1A8	AAB8425	$_{84}$---**ED**LD**REFM**D**FA**D**AQWKAQ**VR**S**L**FSL**FL**SSS**N**G**-$_{115}$	68	94
UGT1A9	NP_066307	$_{84}$---**ED**LD**REF**KA**FAHAQWKAQ**VR**SI**Y**SLLM**G**S**YND-$_{115}$	65	93

Multiple sequence alignment was performed with the Clustal W2 program (http://www.ebi.ac.uk/Tools/msa/clustalw2/) for amino acid residues 84-115 of UGT1A10, which were used as an antigen in this study. Amino acids that are identical to those of UGT1A10 are typed in bold letters.

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Table 3. Sequence alignment of the antigen peptide of human UGT1A10 with highly similar rodent Ugt1a isoforms

Isoform	Accession No.	Sequence	Identity with UGT1A10 (%)	
			84-115	Full-length
Human UGT1A10	AAB81537	$_{84}$EDQNREFMVFAHAQWKAQAQSIFSLLMSSSSG$_{115}$	-	-
Mouse Ugt1a7c	AAP48598	$_{84}$**ED**L**NREF**KISID**AQWK**S**Q**QEGGILP**L**LD**S**PAK$_{115}$	40	75
Mouse Ugt1a9	AAP48599	$_{82}$**ED**LD**REF**KYLSYT**QWK**TPEH**SI**R**S**F**L**TG**S**AR**G**$_{113}$	43	78
Mouse Ugt1a10	AAP48600	$_{84}$**ED**LD**REF**KY**F**TYT**QWK**TPE**QSI**R**S**FMTG**S**AR**G**$_{115}$	46	76
Rat Ugt1a7	AAR95635	$_{84}$**ED**LN**REF**KF**F**IDS**QWK**T**Q**QEGGVLP**L**LT**S**PAQ$_{115}$	40	75
Rat Ugt1a8	AAR95636	$_{84}$**ED**L**N**YH**F**KF**FAH**N**QWK**T**Q**EVGM**FSLL**KH**S**GK**G**$_{115}$	53	75
Rat Ugt1a10	AAR95630	$_{84}$**ED**MD**REF**KH**F**SYT**QWK**TPE**QS**MY**SL**ITG**S**VKD$_{115}$	43	73

Multiple sequence alignment was performed with the Clustal W2 program (http://www.ebi.ac.uk/Tools/msa/clustalw2/) for amino acid residues 84-115 of UGT1A10 and those of the corresponding region of rodent Ugt1a isoforms. Amino acids that are identical to those of UGT1A10 are typed in bold letters.

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Fig. 1

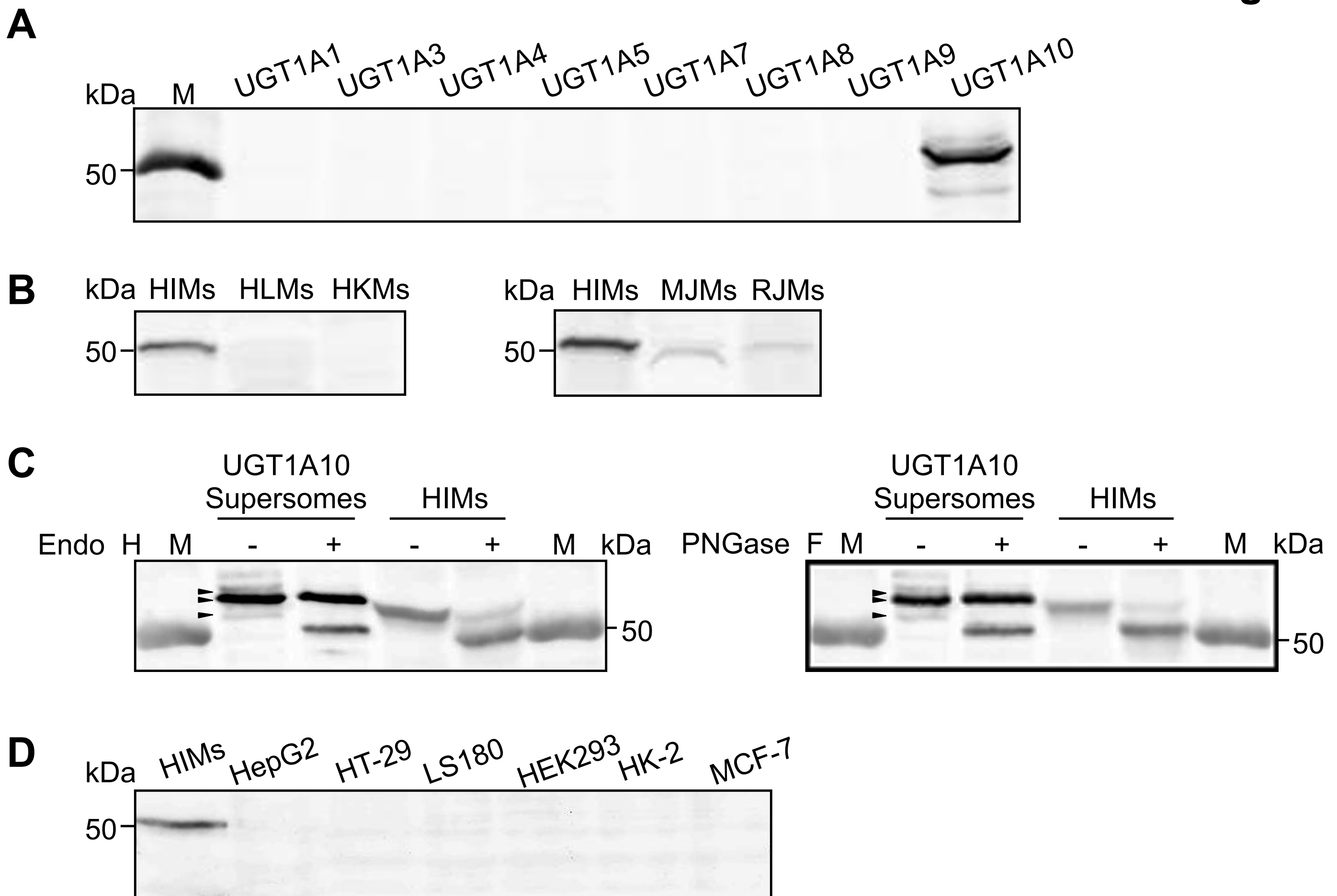

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Fig. 2

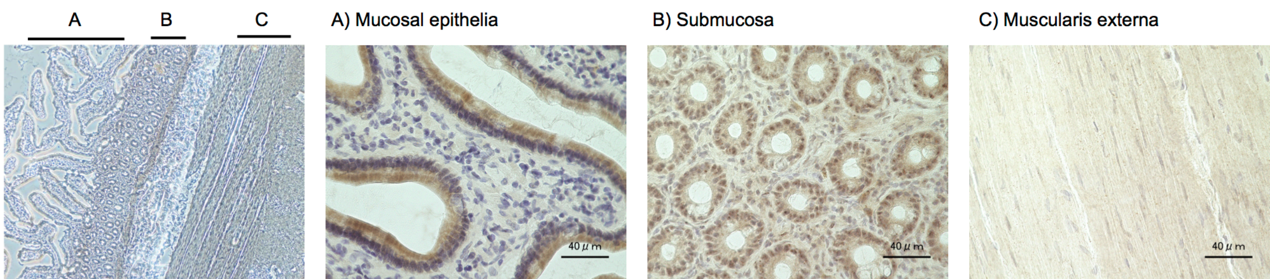

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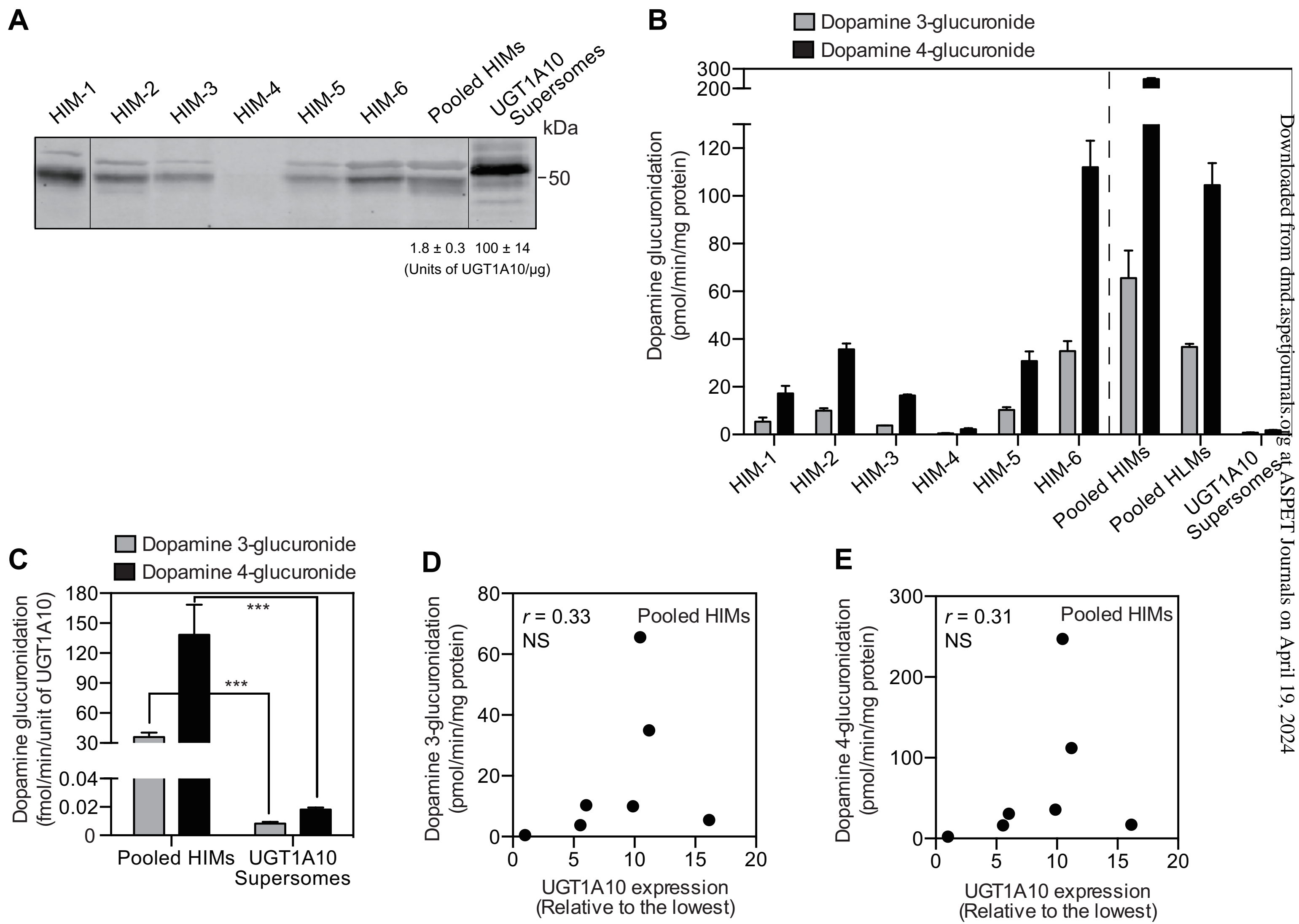
Fig. 3



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