Immunohistochemical expression of conjugating UGT1A-derived splice proteins in normal and tumoural drug-metabolising tissues in humans.

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Short Running Title: Expression of UGT1A in human tissues

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ABSTRACT

Glucuronidation by UDP-glucuronyltransferase (UGT) enzymes is the prevailing conjugative pathway for the metabolism of both xenobiotics and endogenous compounds. Alterations in this pathway, such as those generated by common genetic polymorphisms, have been shown to significantly impact on the health of individuals, influencing cancer susceptibility, responsiveness to drugs, and drug-induced toxicity. Alternative usage of terminal exons leads to UGT1A-derived splice variants, namely the classical and enzymatically active isoforms 1 (i1) and the novel enzymatically inactive isoforms 2, or i2. *In vitro* functional data from heterologous expression and RNA interference experiments, indicate that these i2 isoforms act as negative modulators of glucuronidation, likely by forming inactive complexes with active isoform 1. We used specific antibodies against either active i1 or inactive i2 proteins to examine their distribution in major drug-metabolising tissues. Data revealed that UGT1A_i1 and inactive UGT1A_i2 are co-produced in the same tissue structures, including liver, kidney, stomach, intestine, and colon. Examination of the cellular distribution and semiquantitative level of expression of UGT1As revealed heterogeneous expression of i1 and i2 proteins, with increased expression of i2 in liver tumours and decreased levels of i1 and i2 in colon cancer specimens, compared to normal tissues. These differences in expression may be relevant to human colon and liver cancer tumourigenesis. Our data clearly demonstrate the similar immunolocalisation of active and inactive UGT1A isoforms in most UGT1A-expressing cell types of major tissues involved in drug metabolism. These expression patterns are consistent with a dominant-negative function for the i2 encoded by the UGT1A gene.

Key words: UDP-glucuronosyltransferase (UGT), splice variants, dominant negative function, human tissues, metabolism.

INTRODUCTION

A large number of compounds from environmental and dietary sources that contribute to carcinogenesis are metabolised by a major conjugative pathway mediated by UDP-glucuronosyltransferase (UGT) enzymes [1]. This process is especially active in the liver, but also in other excretory organs, such as kidney, small intestine, and colon. The human *UGT1A* gene encodes multiple enzymatically functional proteins due to the use of alternative promoters and first exons. Recently, novel UGT1A-associated proteins named isoforms 2 (i2), derived from alternative splicing of the terminal exon were described [2, 3]. Functional data reveal that these shorter enzymatically inactive UGT1As act as dominant-negative modulators of glucuronidation activity through protein-protein interactions [2, 3], suggesting an autoregulatory mechanism for UGT1A_i1 function based on UGT1A_i2 expression. In support of the latter assumption, suppression of i2 endogenous splice variants by RNA interference enhances glucuronidation activity in colon cancer cell lines, supporting a dominant-negative effect for i2 products in human cancer cells [4]. As a result, the relative abundance of splice variants would likely impact on glucuronidation activity, potentially contributing to interindividual variability in UGT1A activity and perhaps cancer susceptibility [3]. This phenomenon relies on the simultaneous expression of splice forms in the same cell. Although we have observed the expression of both i1 and i2 isoforms in protein extracts and microsomal preparations from human tissues and cell lines, the relative distribution of these isoforms in specific tissue structures and cell types is still unknown. In fact, only limited data are available on the expression and localisation of UGT1A proteins in human tissues, most likely due to the lack of specific antibodies (Ab) [5-9].

Our current study was designed to investigate the cellular distribution of UGT1A isoforms in human tissues that significantly participate in glucuronidation, namely the liver, kidney, and gastrointestinal tract. Samples from various individuals were immunohistochemically stained using specific

polyclonal Ab to individually detect the i1 and i2 UGT1A spliced variants, whereas additional Ab were used to further document the general or specific expression patterns of UGT1A family members. Given the significant variability due to alternative splicing, especially in cancer [10, 11], we also examined whether the UGT1A splicing pattern is altered in cancer tissues derived from liver and colon.

MATERIAL AND METHODS

Novel polyclonal Ab for enzymatically active and inactive UGT1A isoforms.

Anti-UGT1A_i2 Ab was raised against a peptide corresponding to the amino acid residues encoded by terminal exon 5b (RKKQQSGRQM), which is exclusive to i2. Several rabbits were injected with a total of 100 μg of purified peptide coupled to keyhole limpet hæmocyanin (KLH) as a carrier protein, in presence of incomplete Freund's adjuvant. Antibody production was assessed 12 d after the injections. Polyclonal rabbit Anti-UGT1A_i1 Ab were developed by Genscript (Piscataway, NJ) using a synthetic peptide localised in exon 5a (CKKGRVKKAHKSKTH) conjugated to KLH carrier protein (Table 1). The specificity of these Ab was assessed using complete serum in Western blot experiments performed on microsomal extracts obtained from cells stably expressing either UGT1A_i1 or i2, or both isoforms (Figure 1). Sera #9348 and #4863 were immunoreactive exclusively for isoforms i1 and i2, respectively. Anti-human UGT1As RC-71 was used as a positive control [12].

Clinical and pathological analysis of human tissue samples

Paraffin blocks from 19 individuals were available. All patients provided written informed consent for experimental purposes, and the Institutional Review Boards approved the use of these samples. Independent samples of five types of tissues were evaluated from different individuals: liver (n = 4),

kidney (n = 4), stomach (n = 4), small intestine (n = 4), and colon (n = 3). Corresponding malignant liver and colon samples were further evaluated.

Immunohistochemistry in human tissue specimens

Immunohistochemistry experiments were performed as described [4], using anti-human UGT1A RC-71 (1:250) [12], anti-i1 (#9348; 1:250), anti-i2 (#4863; 1:250) [4], anti-UGT1A1 (1:500) [13], and anti-UGT1A8/1A9 (1:250) [14] for immunostaining of UGT1A proteins. The intensity of the staining was controlled under the microscope. Non-immune serum was used as a negative control, which showed no immunoreactivity in any tissue. Evaluation of staining was done independently by 3 observers, namely 2 pathologists (G.P. and I.P.) and one researcher (J.B.). UGT expression was assessed by semiquantitative scoring of the intensity of staining and recorded as absent (0), weak (1+), moderate (2+), or strong (3+).

RESULTS

Distribution of UGT1A protein isoforms in healthy human tissues

We first evaluated the tissue distribution of human UGT1A protein isoforms by assessing the presence of UGT1A isoforms with the polyclonal Ab RC-71. We then looked for the expression of selected UGT1A enzymes for which specific Ab are available, namely UGT1A1 (Ab #518) as well as UGT1A8 and UGT1A9 (Ab #519). Expression of UGT1A i1 and i2 isoforms was then visualised using Ab that specifically react with UGT1A_i1 (Ab #9348) and UGT1A_i2 (Ab #4863) proteins (Figure 1). We analysed tissue specimens from human liver, kidney, stomach, small intestine, and colon. Results from this immunohistochemical study are summarised in **Table 2**.

In normal liver tissues, staining patterns for UGT1As were of moderate intensity (2+) in hepatocytes, with the strongest expression in hepatocytes lining the centrilobular vein (Figure 2A; Ab RC-71). Of the portal triad, only bile ducts appeared to be immunoreactive, whereas hepatic artery and portal vein walls did not display any immunoreactivity. Similarly, for all other Ab, strong reactivity was observed in hepatocytes and bile ducts, suggesting colocalisation of i1 and i2 forms and expression of specific UGTs such as UGT1A1, UGT1A8, and UGT1A9. Cells lining the vascular wall of hepatic arteries were positively stained with UGT1A_i1 (#9348) and UGT1A1 Ab (Ab #518), a pattern not observed with other Ab (Figure 2 A-B). In addition, the anti-UGT1A1 Ab further revealed specific immunoreactivity in Kupffer cells (Figure 2B).

Analyses of healthy kidney tissues revealed a strong but diffuse expression of UGT1As in the cortical region, with a progressive decrease along the medulla (Figure 3; Ab RC-71). Proximal convoluted tubules were the most strongly immunoreactive to Ab RC-71 (UGT1As) (3+) as compared to distal convoluted tubules (1+), whereas expression in collecting ducts of the medulla

was much more variable among samples (1+–3+). Expression of UGT1As in the loops of Henle ranged from very weak to moderate, whereas no immunoreactivity was seen in the glomeruli or Bowman's capsule, as reported by Gaganis *et al.* [5]. We also noted positive immunostaining for UGT1As in smooth muscle cells of the renal arterial wall (1+) and similar expression patterns was with the other Ab (Figure 3).

Gastric tissues showed widespread expression of UGT1As (Figure 4; Ab RC-71). The mucosa displayed the strongest reactivity, with a decreasing intensity towards the surface. Intense staining was also found in the lymph nodes (3+), in cells of the muscularis mucosae (2+/3+), and in the vascular wall (1+–3+). This expression profile was also noted with the other Ab, except for anti-UGT1A1, which gave only weak immunoreactivity in the mucosa.

In small intestine tissue sections, immunohistochemical staining for UGT1As showed strong expression in the majority of intestinal glands and absorptive cells on the surface of villi (2+) (Figure 5A; Ab RC-71). Intestinal crypts exhibited equivalent staining among all samples (1+ to 2+). Immunoreactive UGT1A proteins were also observed in cells lining the vascular wall (1+ to 2+) (Figure 5B) and in lymph node cells (2+). Strong immunoreactivity toward anti-i1, anti-UGT1A1, and anti-UGT1A8/9 Ab was detected in most epithelial cells, glands, and vasculature. In contrast, uniformly weak staining was seen in similar structures with anti-i2 specific antiserum (Figure 5A), suggesting that active UGT1A_i1 proteins were predominantly expressed in normal intestinal tissues compared to inactive i2. In addition, strong and specific staining with anti-UGT1A and anti-i1 Ab (3+) was observed in Paneth cells, which are found at the bottom of Lieberkühn crypts, and are characterised by large apical eosinophilic granules and a specific role in antimicrobial defense, whereas barely detectable staining was observed with anti-i2 (Figure 5A & B). Interestingly, a

particularly strong signal was detected in the lamina propria of the mucosa using Ab specific to UGT1As and UGT1A1, suggesting an enhanced expression of UGT1A1_i1 in this layer of the mucosa (Figure 5A).

In human colon specimens, epithelial cells lining the intestine wall were strongly reactive to anti-UGT1A RC-71, decreasing in intensity along the crypt (Figure 6). UGT1As were widely expressed in smooth muscle cells, lymph nodes, and cells lining the vascular wall. Anti-UGT1A_i1 (#9348) and anti-UGT1A_i2 antisera (#4863) revealed a similar expression profile, in further support of a strong expression of UGT1A_i1 and _i2 in these tissues. Antibodies against UGT1A1 and UGT1A8/9 yielded expression profiles similar to RC-71 (which reacts towards all UGT1As). Interestingly, as observed in small intestine samples, specific staining of the lamina propria was detected with anti-i1 and anti-UGT1A1, suggesting the expression of the UGT1A1_i1 isoform in this layer of the intestinal mucosa.

Our findings clearly demonstrated a colocalisation of both the active and inactive UGT1A isoforms in most UGT1A-expressing cell types. Altogether, our data are consistent with previous studies on the cellular distribution of enzymes in the tissues analysed above, and, for the first time, report additional UGT1A-positive structures such as Kupffer cells in the liver and smooth muscle cells in the renal arterial wall.

Immunolocalisation of UGT1A in tumour samples

Considering that tumour induction and progression are frequently associated with aberrant splicing, we addressed the question whether the active and inactive UGT1A isoforms are differentially expressed in cancer specimens, especially in liver and colon cancer.

Staining for UGT1A_i1 was stronger in normal and peritumour hepatocytes compared to tumour cells (Figure 7). In contrast, UGT1A_i2 appeared to be highly expressed in cancer cells with a progressive reduction in peritumour and normal hepatic cells (Figures 7 and 8), suggesting a differential regulation of the various spliced isoforms during tumourigenesis. A strongly reduced immunoreactivity toward specific Ab against UGT1A1 and UGT1A8/UGT1A9 was also observed at tumour sites compared to normal and peritumour tissues (Figure 7).

In colon cancer specimens, peritumour structures adjacent to tumour sites displayed expression patterns comparable to healthy tissues for all Ab (Figure 9), with the strongest staining mainly present in epithelial cells. On the other hand, tumour cells showed a decrease in both UGT1A_i1 and _i2 expression, but with a marked heterogeneity in expression levels among tumour samples, ranging from fully negative to moderately stained. These results are in agreement with previous observations showing a generalised decrease in UGT1A expression in colon carcinomas. Our results further suggest that a downregulation of UGT1A expression might represent an early event in tumourigenesis [7].

DISCUSSION

One of the most prominent observations in this study is the finding of a co-expression of both enzymatically active UGT1A_i1 and inactive UGT1A_i2 isoforms in most healthy tissue structures. Such a coexpression is consistent with the dominant-negative function previously proposed for UGT1A_i2 proteins [4], given their colocalisation with UGT1A_i1 in the same cell types. This hypothesis is further supported by independent experiments using the heterologous co-expression of both spliced forms in human cells, as well as small interfering RNA–mediated suppression of endogenous UGT1A_i2 proteins in human colon cancer cells [4].

We demonstrated a strong and widespread expression of both UGT1A_i1 and UGT1A_i2 proteins in most hepatic cells and bile ducts. We also found a specific immunodetection of UGT1A_i1 isoforms in hepatic arteries, which was also observed using the anti-UGT1A1 Ab, supporting the presence of UGT1A1_i1 in these vascular structures. Similarly, the expression of other UGT1As such as UGT1A6 along the vascular wall of hepatic arteries has been reported in rat liver, and altogether, these observations suggest the presence of active enzymes in the endothelium of hepatic arteries [8]. Kupffer cells appeared strongly immunoreactive to anti-UGT1A1 in both normal and malignant tissues. A protective role has been previously proposed [15] [16, 17] for Kupffer cells, which play a role in the production of bilirubin from heme and represent 80–90% of all fixed macrophages in the whole body. Moreover, one study suggested that Kupffer cells are involved in the regulation of paracetamol metabolism by glucuronidation [18].

Drugs that are excreted in urine may be metabolized in the kidney. Recent studies support a role for UGT enzymes in metabolic processes of renal elimination, and the presence of both phase I and II detoxification systems has been established in kidney [19, 20]. The wide expression of UGT1As in

the kidney is consistent with the renal functions in detoxification and excretion. Although the absence of UGT1A1-mediated activity in kidney microsomes is well documented, the positive immunostaining of renal structures with anti-UGT1A1 can be attributed to UGT1A1_i2. This is supported by the findings that anti-UGT1A1 also reacts with UGT1A1_i2 and that i2 is strongly expressed in kidney protein and mRNA preparations [2, 3]. However, the role of i2 variants in kidney in the absence of the active UGT1A1_i1 remains unclear.

The expression of UGTs in proximal convoluted renal tubules [5, 21] suggests a protective role for glucuronidation because proximal convoluted tubules are located next to glomeruli where they are initially in contact with the glomerular filtrate. Several substrates of UGTs are produced at multiple sites within the kidney, including the glomerular and vascular endothelia, the medullary tubules, the cortical collecting tubules, and the Henle's loop [22]. These substrates include arachidonic acid derivatives such as eicosanoids, which exert diverse and complex functions. In addition to their role in normal kidney function, these lipids play important roles in the pathogenesis of kidney-related diseases, such as hypertension and acute kidney injury caused by ischemia/reperfusion and toxic insults [23]. Eicosanoids have been shown to undergo glucuronidation by UGT1A enzymes, a finding consistent with a role for UGTs in regulating renal activity [24]. The presence of regulating i2 isoforms could therefore provide an additional level in the regulation of the UGT1A pathway, as further supported by overexpression and siRNA approaches.

In addition to liver and kidney, UGTs are highly expressed in the gastrointestinal tract, where they have the potential to impact on metabolism and on the physiological effects of ingested xenobiotics or nutrients [25-27]. An ubiquitous, albeit variable expression of active UGT1A_i1 was detected throughout the gastrointestinal tract. Stomach and colon specimens strongly expressed UGT1A_i2

splice variants, concurrently with active UGT1A_i1. A strong expression was observed in the stomach, mostly in parietal and principal cells of the gastric glands, which produce hydrochloric acid and various gastric enzymes such as pepsinogen and gastric lipase. These results contrast with observations made by Peters *et al.* (1987) using tools that did not distinguish between UGT1A splice variants, who showed positive immunostaining of the surface epithelium but no detectable staining in gastric glands [21]. Interestingly, some differences were observed between the expression profile of i1 and i2 isoforms in gastric tissues. While i1 variants are not expressed in most of the outer cell layer of the stomach, anti-i2, anti-1As and anti-1A1 Ab display a similar and strong reactivity with this particular layer. These observations suggest a preferential expression of inactive UGTs (e.g. 1A1_i2) over active i1 variants in this specific layer of the stomach. Moreover, i2 isoforms appear to be mainly concentrated within granules of principal cells, most likely zymogen granules, whereas anti-UGT1A_i1-associated staining is more homogenously dispersed in these cells. The latter observation indicates that both variants do not always co-localise in similar cellular structures. Accordingly, whether i2 variants might exert roles additional to their putative function as UGT1A inhibitors has yet to be established. Our previous work revealed numerous interactions between UGTs [3, 28, 29], and other reports support potential protein-protein interactions between UGTs and non-UGT proteins [30].

We also report for the first time a strong expression of active UGT1A_i1 isoforms in Paneth intestinal cells. The location of Paneth cells in close proximity to stem cells suggests that they play a critical role in epithelial cell renewal. Protection of these stem cells is essential for the long-term maintenance of the intestinal epithelium [31]. Also, the strong immunoreactivity with anti-UGT1A_i1 and anti-UGT1A1 in the lamina propria of intestinal mucosa of the small intestine and colon tissue samples implies a strong expression of UGT1A1_i1. Since the mucosal epithelium is

relatively delicate and vulnerable (i.e. it can easily be breached by potential invading microorganisms, compared to the epidermis), the lamina propria contains numerous cells with immune functions that provide an effective first line of defence.

The mRNA splicing process is frequently disrupted during tumourigenesis, leading to the induction of alternative splicing events [11]. Therefore, a higher level of UGT1A_i2 expression in tumour samples might be expected, as illustrated in the liver specimens tested. Others have reported a stable expression of UGT1As, without distinction between splice variants, in benign liver tumours such as focal nodular hyperplasia [32]. Another study reported a downregulation of several UGT1As, including UGT1A1, UGT1A3, UGT1A4, and UGT1A9, but not UGT1A6, in pre-malignant and malignant liver tissues (e.g., hepatic adenoma and hepatocellular carcinoma) [32]. The changes observed in the expression levels of various UGT1As were further shown to be specific to the type of tumour, e.g. UGT1A1 was significantly reduced in hepatocellular carcinoma but not in cholangiocellular carcinoma, whereas UGT1A4 was significantly decreased in both types of cancer [32]. Whether this decrease in UGT1A expression levels might be a consequence of changes in i1/i2 ratios remains to be determined, but only barely detectable levels of UGT1A6_i2 mRNA have been observed in liver samples [2], consistent with the stability of expression of this particular isoform in cancer. Interestingly, liver cancer specimens displayed a differential expression of active UGT1A_i1 and inactive i2 isoforms. Indeed, cancer cells showed stronger UGT1A_i2 expression levels and lower UGT1A_i1 levels, whereas we observed the reverse staining pattern in peritumour and normal liver cells. We hypothesize that under these circumstances, the production of UGT1A_i2 inactive forms predominates over active UGT1A_i1 in cancer cells, substantially reducing glucuronidation activity. Additional studies are required to quantitatively document the expression profiles and functional consequences of variations in the i1:i2 ratio.

We conclude that active UGT1A_i1 and inactive UGT1A_i2 isoforms are co-synthesised in the tissue structures analysed in the present study, providing supporting evidence for the dominant-negative role of UGT1A_i2 variants previously proposed using in vitro approaches. The differential regulation of their expression in cancer might affect the UGT1A-mediated glucuronidation pathway.

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AUTHOR CONTRIBUTIONS

CG designed the study. BT provided human tissue samples. JB and MR carried out experiments and data collection. JB, IP, GP, BT and CG analyzed and interpreted the data. Funding was provided by CG. JB, MH and CG were involved in writing of the paper and all authors had final approval of the submitted version.

LIST OF ABBREVIATIONS

UGT, UDP-glucuronosyltransferase; i1, isoform 1; i2, isoform 2; Ab, antibody; KLH, keyhole limpet hæmatoxylin; DAB, 3,3'-diaminobenzidine.

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Table 1: Specific target sequences of UGT antibodies

Antibody	Region of the protein targeted (a.a.)	Ref
Anti-UGT1As	312-531 (exons 2-5a)	[12]
Anti- UGT_i1	520-533 (exon 5a)	-
Anti- UGT_i2	435-444 (exon 5b)	[4]
Anti- UGT1A1	63-144 (exon 1A1)	[13]
Anti- UGT1A8/UGT1A9	3-118 (exon 1A9)	[14, 33]

TABLE 2 UGT-expressing structures in five human tissues.

Tissues and structures	UGT1A (Ab RC-71)	i1 (Ab #9348)	i2 (Ab #4863, #4864)	1A1 i1&i2 (Ab #518)	1A8/1A9 i1 & i2 (Ab #519)
LIVER					
Hepatocytes	2+*	2+	2+	2+	2+
Portal triad					
Bile ducts	1+	1+	1+	1+	1+
Hepatic arteries	–	1+	–	1+	–
Portal vein	–	–	–	–	–
KIDNEY					
Vasculature	1+	1+	1+	1+	1+
Glomeruli	–	–	–	–	–
Bowman's capsule	–	–	–	–	–
Proximal convoluted tubules	3+	3+	3+	3+	3+
Distal convoluted tubules	1+	1+	1+	1+	1+
Collecting ducts	Δ 1+ to 3+	Δ 1+ to 3+	Δ 1+ to 3+	Δ 1+ to 3+	Δ 1+ to 3+
Loop of Henle	Δ 0+ / 2+	Δ 0+ / 2+	Δ 0+ / 2+	Δ 0+ / 2+	Δ 0+ / 2+
STOMACH					
Mucosa					
Surface epithelium	0+/1+	0+/1+	0+/1+	0+/1+	0+/1+
Gastric glands					
Chief cells	2+/3+	2+/3+	2+/3+	1+	1+/2+
Parietal cells	2+/3+	2+/3+	2+/3+	1+/2+	1+/2+
Crypts	2+	1+/2+	1+/2+	1+/2+	1+
Smooth muscle cells	1+	1+	2+	2+/3+	1+
Lymph nodes	2+	3+	3+	3+	2+
Vasculature	1+	1+	2+	3+	1+
SMALL INTESTINE					
Mucosa					

Surface epithelium:	2+	2+	0+ /1+	1+/2+	3+
Absorptive cells					
Intestinal glands					
Goblet cells	1+	1+/2+	0+ /1+	0+ /1+	1+
Paneth cells	3+	3+	0+ /1+	0+ /1+	3+
Crypts					
Goblet cells	1+	2+	0+ /1+	0+/1+	2+
Paneth cells	3+	2+/3+	0+ /1+	0+/1+	
Lamina propria	–	3+	–	3+	–
Vasculature	3+	3+	0+ /1+	2+/3+	3+
Smooth muscle cells		3+	1+	1+/2+	2+
COLON					
Mucosa					
Surface epithelium:	3+	3+	3+	3+	3+
Absorptive cells					
Intestinal glands	1+	0+/1+	1+	1+	0+/1+
Crypts	1+/2+	1+/2+	1+/2+	1+/2+	1+
Lamina propria	–	3+	–	3+	–
Vasculature	2+	3+	2+	2+	3+
Smooth muscle cells	2+	3+	1+	2+	1+
Lymph nodes	2+		2+	2+	

*The staining intensity was recorded as absent (–), barely detectable (0+), weak (1+), moderate (2+), or strong (3+).

FIGURE LEGENDS

Figure 1. Specificity of polyclonal anti-UGT1A_i1

Specificity of novel polyclonal anti-i1 (Ab #9348) assessed by Western blot analysis. Microsomal proteins from HEK293 cells stably expressing UGT1As, including UGT1A9_i1, 1A9_i1+i2, and 1A9_i2, were separated on 10% SDS-PAGE gels and tested. Specificity of the Ab towards other UGT1As was tested but is not shown. Anti-i1 (dilution $1:10^5$) binds only to isoform 1 proteins and not to isoform 2 or to HEK293 cells (negative control).

Figure 2. UGT1A isoforms 1 and 2 are widely expressed in human liver tissues.

A) Immunohistochemistry experiments were performed on four normal human samples using human anti-i1 (#9348), anti-i2 (Ab #4863 and #4864), anti-UGT1A1 (Ab #518), and anti-UGT1A8/1A9 (Ab #519). Anti-human UGT1As RC-71 was used as a reference to document expression of all UGT1As. No staining was detected using nonimmune serum as a negative control followed by incubation with the secondary antibody. Typical results for a specimen of normal liver tissue stained with all Ab are shown. B) Highly magnified images of 1A1-positive structures are depicted (Ab #518; 40× objective). Positive structures are identified by black arrows. BD, bile ducts; HA, hepatic arteries; PV, portal vein.

Figure 3. Immunohistochemistry of cortical and medullary sections of human kidney reveals strong expression of UGT1As. Human kidney sections (n = 4) were incubated overnight with the six Ab described in Figure 2. Negative controls were performed using nonimmune serum. Typical results for a specimen of normal kidney tissue are shown. A, arterial wall; D, distal

convoluted tubules; P, proximal convoluted tubules; C, collecting ducts; G, Glomerulus; LOH, Loop of Henle.

Figure 4. I1 and i2 spliced forms are expressed together in human gastric tissues. Immunohistochemistry experiments were performed on four human gastric samples using specific Ab. No staining was detected when nonimmune serum was used as a negative control. Typical results are shown for a specimen of normal gastric tissue stained with all Ab.

Figure 5. Expression of alternatively spliced forms in intestinal tissues. A) Sections of intestinal sections are shown (n = 4) stained with the six Ab tested. B) Highly magnified images of intestinal structures and cell types are depicted (Ab RC-71; 40× objective). Negative controls are illustrated. Typical results for a specimen of normal intestinal tissue are shown. Gl, gland; A, artery; V, vein.

Figure 6. Colon tissues express high levels of both active and inactive UGT1A spliced isoforms. Three colon samples were analysed for their expression of UGT1As using the six Ab described in Figure 2. No staining was detected in negative controls. Typical results are shown for a specimen of normal colon tissue.

Figure 7. Heterogeneous expression of UGT1As in human liver tumour tissues. Immunohistochemical analysis of four tumour sections and adjacent normal peritumour tissue was performed with Ab specific for UGT1A_i1 (Ab #9348), i2 (Ab #4863 and #4864), 1A1 (Ab #518), and 1A8/1A9 (Ab #519).

Figure 8. Increased expression of UGT1A isoforms 2 in tumour liver samples. Immunohistochemical analysis of four tumour sections and adjacent normal tissues was performed with specific antibodies targeted against i2 proteins. Tumour samples consisted of two hepatocellular carcinomas and two metastatic adenocarcinomas.

Figure 9. UGT1A expression is reduced in colon tumoral tissues. Immunohistochemical analysis of three adenocarcinoma samples and adjacent healthy peritumour tissue was performed with Ab specific for UGT1A_i1, i2, 1A1, and 1A8/1A9.