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Isolation and Characterization of a Human Orphan UDP-Glucuronosyltransferase, UGT2B11

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Glucuronidation is an important metabolic pathway for both endogenous and exogenous compounds. To isolate novel UGT2B cDNA clones, human prostate and LNCaP cell cDNA libraries were screened using a pool of steroid-specific UGT2B cDNA as probes. We have isolated a novel human cDNA of 1.7 kb in length containing an open reading frame of 1587 pb which encodes a deduced protein of 529 residues named UGT2B11. UGT2B11 share 91% identity in amino acids with UGT2B10, a UDP-glucuronosyltransferase (UGT) protein with unknown function. In agreement with other characterized UGT2B proteins, a Western blot analysis showed high levels of a 52-kDa protein present in a microsome preparation from HK293 cells stably transfected with the UGT2B11 cDNA. Despite the screening of 100 potential substrates, glucuronidation activity was not detected for the stably expressed UGT2B11 protein. However, UGT2B11 specific RT-PCR analysis revealed expression of the transcripts in a wide range of human tissues including the liver, kidney, mammary gland, prostate, skin, adipose, adrenal, and lung. The biological function of the UGT2B11 protein is unknown but its wide expression in human tissues raises the possibility that UGT2B11 may constitute an orphan UGT enzyme whose substrates specificity remain to be identified.

Key Words: **orphan UGT; prostate; LNCaP; UGT2B.**

The UDP-glucuronosyltransferase (UGT) gene superfamily encodes enzymes catalyzing the transfer of glucuronic acid to a wide range of exogenous and endogenous compounds including phenols derivatives, bilirubin, fatty acids and steroids (1, 2). The presence of the glucuronyl group on the parent compound results in a more water soluble molecule which can be excreted into bile or urine (1). To date, human UGT enzymes have been classified into two families (UGT1 and UGT2) which are further redivided into subfamilies regarding sequence homology (3). The UGT1 gene locus contains the necessary exons for 12 UGT1 transcripts including three pseudogenes. From cDNA sequence and examination of the gene locus, each UGT1 gene is composed of a unique first exon and four common exons 2 to 5 (4, 5). The UGT2 family is divided into two subfamilies, UGT2A and UGT2B, where the latter subfamily comprises enzymes conjugating bile salts and steroid hormones. Comparison of the UGT2B cDNA sequences and FISH analysis suggests that these isoenzymes are encoded by independent genes resulting from duplication events of a common ancestral gene (6-8).

In humans, eight cDNAs from the UGT2B subfamily have been isolated including three variants (9-19). Extensive tissue distribution of the human and monkey UGT2B isoenzymes has demonstrated that these enzymes are expressed in the liver as well as in a large number of extrahepatic tissues (9, 13, 19-21). From these cDNAs, UGT2B4 and UGT2B7 are known to be implicated in the conjugation of bile acids, catecholestrogens and phenolic derivatives (15, 16, 19). UGT2B7, UGT2B15 and UGT2B17 are mainly involved in the conjugation of steroid hormones especially androgens and their metabolites (9, 10, 13, 18, 22, 23). On the other hand, the human UGT2B10 cDNA transfected in COS-7 cells exhibited no activity toward the more than the forty potential substrates tested (16). To date, no function has been assigned to this widely distributed human isoenzyme (24).

In the present study, we have characterized a novel cDNA that encodes a novel UGT2B protein subfamily

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This work was supported by the Medical Research council (MRC) of Canada, The Fonds de la recherche en santé du Québec and Endorecherche. Éric Lévesque is holder of a scholarship from the MRC of Canada. Recently, the UGT2B11 protein has been identified as a variant of UGT2B4 (3, 19) and, therefore, this trivial name has been reassigned to the novel human UGT2B protein described in this article. The nucleotide sequence of the UGT2B11 cDNA has been submitted to the GenBank/EMBL Data Bank with Accession Number AF016492.

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member. Despite extensive enzymatic analyses, we were not able to detect any glucuronidation activity using a stable cell line expressing high levels of transcript and protein encoding UGT2B11. Tissue distribution experiments using specific RT-PCR analysis revealed expression of UGT2B11 in a wide range of extrahepatic tissues including the human liver, kidney, mammary gland, prostate, placenta, adipose tissue, adrenal and lung.

MATERIALS AND METHODS

Materials. UDP-glucuronic acid and all aglycons were obtained from Sigma Chemical Co. (St-Louis, MO) and ICN Pharmaceutical Inc. (Quebec, Canada). Radioinert steroids were purchased from Steraloids Inc. (Wilton, NH). [^{14}C]UDP-glucuronic acid (285 mCi/mmol) was obtained from NEN Dupont (Boston, MA) and [α-^{32}P]-dCTP (3000 Ci/mmol) was from Amersham (Ontario, Canada). Geneticin (G-418) and Lipofectin were obtained from Gibco BRL (Ontario, Canada). Protein assay reagents were obtained from Bio-Rad (Richmond, CA). Restriction enzymes and other molecular biology reagents were from Pharmacia LKB Biotechnology Inc. (Milwaukee, WI), Gibco BRL (Ontario, Canada), Stratagene (LaJolla, CA) and Boehringer Mannheim (Indianapolis, IN). AmpliTaq DNA polymerase and Pwo polymerase were from Perkin-Elmer Cetus (Branchburg, NJ) and Boehringer Mannheim (Indianapolis, IN) respectively. Human embryonic kidney 293 cells (HK293) and LNCaP cells were obtained from the American Type Culture Collection (Rockville, MD). Total RNA from human prostate, adrenal, testis, mammary gland, kidney, uterus and lung was purchased from Clontech (Palo Alto, CA).

Human RNA isolation. Total RNA was isolated from human liver, adipose tissue, skin, placenta, benign prostate hyperplasic tissue (BPH) and LNCaP cells according to the Tri reagent acid phenol protocol as specified by the supplier (Molecular Research Center Inc., Cincinnati, OH). The mRNAs obtained from a human BPH tissue and LNCaP cells were obtained by affinity chromatography through oligo (dT)-cellulose (Pharmacia, Milwaukee, WN).

cDNA isolation. Affinity purified BPH and LNCaP cell mRNAs were used to construct cDNA libraries in the λ/ZAP express vector as specified by the supplier (Stratagene, LaJolla, CA). Both libraries were not amplified for screening as previously described (9). UGT2B11 cDNA clone was isolated from both libraries and was sequenced in both directions using specific UGT oligonucleotides (9). The UGT2B11 cDNA clones was excised from the pBK-CMV vector.

Stable expression of UGT2B11. HK293 cells were grown in Dulbecco's modified Eagle's medium containing 4.5 g/l glucose, 10 mM hepes, 110 μg/ml sodium pyruvate, 100 IU of penicillin/ml, 100 μg/ml of streptomycin and 10% fetal bovine serum (FBS) in a humidified incubator, with an atmosphere of 5% CO_2, at 37°C. Five μg of pBK-CMV-UGT2B11 was used to transfect HK293 cells using Lipofectin and stable transfectants were selected in media containing 800 μg/ml G-418 as previously reported (9).

Western blot using human UGT2B (EL 93) antibody. Microsomal proteins from HK293 cells and HK293 cells stably expressing UGT2B11 was separated by a 12 % SDS-PAGE gel. The gel was transferred onto a nitrocellulose membrane and probed with the EL 93 anti-UGT2B17 antisera (1:2000 dilution) as reported (13). An anti-rabbit IgG horse antibody conjugated with peroxidase (Amersham, Ontario, Canada) was used as the second antibody and the resulting immunocomplexes were visualized using a chemiluminescence kit (ECL) (Renaissance, Quebec, Canada) and exposed on hyperfilm for 1 hr (Kodak Corp.; Rochester, N.Y.).

Glucuronidation assay using cell homogenates. HK293 cells expressing exogenous UGT2B11 were suspended in Tris buffered saline (9, 25) containing 0.5 mM DTT and homogenized using a Brinkman polytron. Enzyme assays were performed as previously described (9).

Reverse transcriptase polymerase chain reaction (RT-PCR) analysis. The tissue-distribution of UGT2B11 was achieved using a RT-PCR technique as previously reported (9). The antisense primer used for the reverse transcriptase reaction was 5′-CTGATCCCACTTCATATCAGAC-3′. The PCR reaction was carried out by adding 100 pmol of the specific sense primer 5′-TTCCATTCTTTTTGATCCCAATGATG-3′ and 100 pmol of the specific antisense primer 5′-TAGGTATGTAGGAAGGAGGGAAAATC-3′. All PCR reactions were controlled using specific oligonucleotides for GAPDH. The identity of all PCR products was verified by direct sequencing (26).

RESULTS

Isolation of the human UGT2B11 cDNA. To isolate novel cDNA encoding human UGT isoenzymes, a LNCaP cell line and a human prostate cDNA library were screened with a pool of radiolabelled cDNA probes synthesized from the human UGT2B7, UGT2B10 and UGT2B15 cDNAs. Due to the high homology between the UGT2B cDNA clones isolated to date, it was expected that a pool of UGT2B cDNA probes would hybridize to previously isolated clones as well as novel UGT2B cDNAs. Screening approximately 10^6 recombinants yielded 30 positive clones from the LNCaP cell cDNA library and 5 clones from the human prostate cDNA library. Among these positives clones we identified cDNAs encoding UGT2B10 (16) UGT2B15(D^{85}) (10, 18), UGT2B15(Y^{85}) (13), UGT2B17 (9), UGT2B4(E^{458}) (19), UGT2B4(D^{458}) (11, 12, 16) and three UGT2B11 cDNA with identical coding sequences. The longest UGT2B11 cDNA is 1722 bp in length and contains an open reading frame of 1587 flanked by a 5′-untranslated region of 9 bp and a 3′-untranslated region of 123 bp. As found in other UGT2B enzymes, UGT2B11 contains a predicted leader sequence from amino acids 1 to 23 characterized by a charged lysine residue at position 4 and terminates with a possible cleavage site at a cysteine residue at position 23 (27). As well, UGT2B11 has a hydrophobic putative transmembrane region between amino acids 493 to 509 which is followed by a positively charged lysine residue (28). UGT2B11 contains one potential asparagine-linked glycosylation site (NX(S/T) present at amino acid residues 315-317 (Fig. 1).

Characterization of UGT2B11. Amino acid sequence alignment shows that UGT2B11 is 91% identical with UGT2B10 isolated from a human liver cDNA library (16). The UGT2B11 protein share 76-91% identity with the other human UGT isoforms (Table 1). Interestingly, the UGT2B11 shares only 76% identity with the two androgen metabolizing enzymes UGT2B15 and UGT2B17. In every case the amino-terminal region of the protein between residues 1 and 290 is less homologous to UGT2B11 than the carboxyl-terminal region between residues 291

FIG. 1. Amino acid sequence of UGT2B11 and alignment with the sequence of UGT2B10. The amino acid sequence was deduced from the nucleotide sequence. The putative membrane-anchoring domain is indicated by the solid line. The bold residues identify the potential asparagine-linked (NX(S/T)) glycosylation site.

and 530 (Table 1). To demonstrate the expression of the UGT2B11 protein in stably transfected HK293 cells, western blot analysis was performed on microsomal preparations using a UGT2B specific human polyclonal antibody (EL-93) (13). The expression of exogenous UGT2B proteins was demonstrated by the presence of a 53 kDa protein. This protein was absent in the untransfected HK293 cells microsome preparation (Fig. 2). Of the over 100 substrates tested (Table 2), it appears that two UGT2B11-HK293 stable cell lines did not express any detectable glucuronosyltransferase activity.

Tissue distribution of UGT2B11. We performed a specific reverse transcriptase-polymerase chain reaction (RT-PCR) analysis. The oligonucleotides designated for RT-PCR experiment were specific for UGT2B11 and designed not to amplify other UGT2B transcripts isolated to date. To confirm the identity of the amplified 408 bp RT-PCR product, the DNA was analyzed by direct sequencing. It was possible to demonstrate expression of UGT2B11 in liver, kidney, mammary gland, adipose, skin, prostate, benign prostate hyperplasic tissue (BPH), adrenal, lung, LNCaP cells,

TABLE 1

Homology between the Deduced Amino Acid Sequence of UGT2B11 and Other UGT2B Isoenzymes

		UGT2B11		
	Steroid substrates	Amino-terminal domain (1–290) identity (%)	Carboxy-terminal domain (291–530) identity (%)	Overall identity (%)
UGT2B4	4OHE$_1$, E$_3$, 3α-diol	80	89	84
UGT2B7	4OHE$_1$, E$_3$, ADT, 3α-diol	80	92	85
UGT2B10	N.D.	86	96	91
UGT2B15	3α-diol, DHT, T	70	84	76
UGT2B17	DHT, ADT, 3α-diol, T	70	84	76

Note. The sequence identity (%) of the amino-terminal domain from residue 1 to 290, the carboxy-terminal domain from residue 291 to 530, and the entire protein are as indicated. The steroid specificity of each type of enzymes is indicated. N.D. indicates that no substrate has been identified.

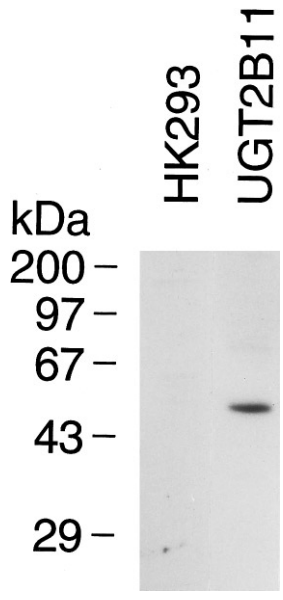

FIG. 2. Immunoblot analysis of HK293 cells stably expressing UGT2B11. Ten μg of microsomal protein isolated from HK293 cells and from HK293-UGT2B11 stable cells were chromatographed on a 10 % SDS-polyacrylamide gel. The gel was transferred and probed with the EL 93 polyclonal antibody.

HepG2 cells and in the HK293-UGT2B11 stable cell line. No PCR product was observed in the negative control samples which contain no cRNA, cRNA from HK293 cells and from HK293 cells expressing UGT2B17 (Fig. 3).

DISCUSSION

We have isolated and characterized a novel human UGT isoenzyme named UGT2B11. The cDNA sequence encodes a protein of 529 amino acids devoid of significant transferase activity. The function of the UGT2B11 protein is unknown, but its wide expression in human peripheral tissues raised several possibilities regarding its biological function. UGT2B11 may constitute an orphan UGT enzyme whose substrates has not been identified. In the UGT2B subfamily, UGT2B11 is the second mRNA encoding a protein without reported transferase activity. The UGT2B10 cDNA isolated from the human liver is highly homologous to UGT2B11, and to date, no functional role as been assigned to this protein (16). The amino acid similarity between the two proteins may indicate a related type of substrate specificity or a common function. It is also clearly established that UGT enzymes exhibit distinct but overlapping substrate specificities, it is possible that UGT2B10 and UGT2B11 share similar substrates having different functional acceptor groups than the compounds used in this study. Recently, as observed for UGT2B10 and UGT2B11, the extrahepatic UGT1A8 enzyme did not demonstrate reactivities toward the substrates tested, and to date, no conjugation function as been assigned to this isoform (29).

In humans, the presence of putative steroid metabolizing enzymes without defined substrates or functions have already been demonstrated. For example, in the human 17β-hydroxysteroid dehydrogenase (17β-HSD) family of enzymes, which are known to be responsible for the interconversion of 17-keto and 17β-hydroxysteroids, it was reported that the protein 17β-HSD type IIB, expressed in several human tissues, has an unresolved biological function since it has no affinity for the steroid hormones tested (30).

On the other hand, it has also been suggested that UGT may form dimer or tetramer *in vivo*. Radiation inactivation analyses indicated that UGT1A1 may act as dimer and tetramer (23). Using the same method, Gschaidmeier and Bock have suggested that monoglucuronidation of phenols may be catalyzed by a dimeric form of UGT while diglucuronidation is catalyzed by a tetramer (29). Two recent reports showed that the amino terminus domain is implicated in the oligomerization process (31, 32). Meech and Mackenzie demonstrated that when two inactive forms of UGT2B1 are coexpressed in cell culture, catalytic activity can be restored (32). Using rat hepatic microsomes Ikushiro *et al.* co-immunopurified UGT2B1 with UGT1s, in addition to others minor species of coeluted proteins, UGT2B3 and UGT2B6 (31). Evidence for direct interaction of UGT2B1 with UGT1s was obtained by the loss of UGT2B1 adsorption to immunoaffinity column in Gunn rat hepatic microsomes, which lack all UGT1 isozymes (31). Knowing that UGT enzymes can exist as oligomers, it is possible that UGT2B11 might form heterodimers with other members of the UGT1 or UGT2 family, thus affecting the transferase activity of these enzymes. It is also conceivable that UGT2B11 needs to be specifically associated with a second UGT enzyme to present an enzymatic activity. The existence of multiple UGT proteins, and the potential associations between different subtypes, may be a mechanism involved in the regulation of transferase activity.

Considering a recent hypothesis by Iyanagi (31), UGT2B11 may also be involved in cofactor transport. Using microsomal vesicles, they demonstrated that UGT complex formation is associated with the UDPGA uptake process stimulated by UDP-GlcNAc. They proposed that dimer and/or higher oligomer forms of UGTs may act as channels for UDPGA access from the cytosolic side to the UGT active site in the cisternal lumen or within membranes. Oligomers formation can implicate UGT2B11 *in vivo*, where this unfunctional protein might regulate the transferase activity of other well charaterized human UGTs by a modulation of the cofactor availability to the active site of the transferase complex.

TABLE 2

List of Compounds That Do Not React with UGT2B11

Endogenous compounds

C_{19} STEROIDS

Testosterone
Dihydrotestosterone (DHT)
Androsterone (ADT)
Epiandrosterone
Dehydroepiandrosterone (DHEA)
Etiocholanolone
Androst-5-ene-3β,17β-diol (Δ5-diol)
5α-androstane-3α,17β-diol (3α-diol)
5β-androstane-3β,17β-diol (3β-diol)
5β-androstane-3α,17β-diol
5α-androstane-3α,11β,17β-triol
5β-androstane-3α,16α,17β-triol
5β-androstane-3α,11α,17β-triol

C_{18} STEROIDS

Estrogens
Estrone (E_1)
Estradiol (E_2)
Estriol (E_3)
Catecholestrogens
1,3,5,10-Estratriene-2,3-diol-17-one
1,3,5,10-Estratriene-3,4-diol-17-one,
1,3,5,10-Estratriene-3,16α-diol-17-one,
1,3,5,10-Estratriene-2,17β-triol,
1,3,5,10-Estratriene-3,4,17β-triol
1,3,5,10-Estratriene-2,3,16α,17β-tetrol,

C_{21} STEROIDS

Progesterone
17-OH-Progesterone
Pregnenolone
17-OH-Pregnenolone
5α-pregnane-3α-ol-20-one
5α-pregnane-3α,20α-diol
5α-pregnane-3α,17α-diol-20-one
5α-pregnane-3β,17α-diol-20-one
5α-pregnane-3β,17α-diol-11,20-dione
5β-pregnane-3α,20α-diol
5β-pregnane-3α,17α-diol-20-one
5β-pregnane-3α,6α,17α-triol-20-one
Aldosterone
Tetrahydroaldosterone

Endogenous compounds

CORTISOL AND METABOLITES

Cortisol
11-deoxycortisol (11-Doc)
Deoxycorticosterone (Doc)
Cortisone
5α-dihydrocortisol
5β-dihydrocortisol
5α-dihydrocortisone
5β-dihydrocortisone
5α-tetrahydrocortisol
5α-tetrahydrocortisol-3β
5β-tetrahydrocortisol
5α-tetrahydrocortisone
5α-tetrahydrocortisone-3β
5β-tetrahydrocortisone

BILE ACIDS

Chenodeoxycholic acid
Cholic acid
Lithocholic acid
Hyodeoxycholic acid (HDCA)

NEUROMODULATORS

Dopamine
Serotonine

OTHERS

Cholesterol
Retinoic acid
T_3
T_4
Vitamine D_3
Vitamine C

Exogenous compounds

COUMARINS

4-methylumbelliferone
Scopoletin

NON STEROIDAL ANTI-INFLAMMATORY DRUGS (NSAID)

Acetyl Salicylic acid
Ibuprofen
Indomethacin
Salicylic acid

Exogenous compounds

ANTHRAQUINONES

Anthraflavic Acid
Quinalizarin

BENZODIAZEPINS

Chlodiazepoxide (Librium)
Diazepam (Valium)
Lorazepam (Ativan)
Oxazepam (Serax)

FLAVONOIDS

5,7-dihydroxyflavone
7-hydroxyflavone
Naringenin

MONOTERPENOID

Borneol

OPIOIDS

Codeine
Morphine
Naltrexone

OTHER DRUGS

4-acetaminophenol
Chloramphenicol
17β-ethinylestradiol
Hydroxyflutamide
Furosemide

SAPOGENIN

Hecogenin

PHENOLIC COMPOUNDS

4-ethyl-phenol
4-propyl-phenol
4-T-buthyl-phenol
4-aminophenol
o,o′-biphenyl
p,p′-biphenyl
Eugenol
1-naphtol
p-nitrophenol
Phenolphtalein
Phenol red

OTHERS

Imidazol

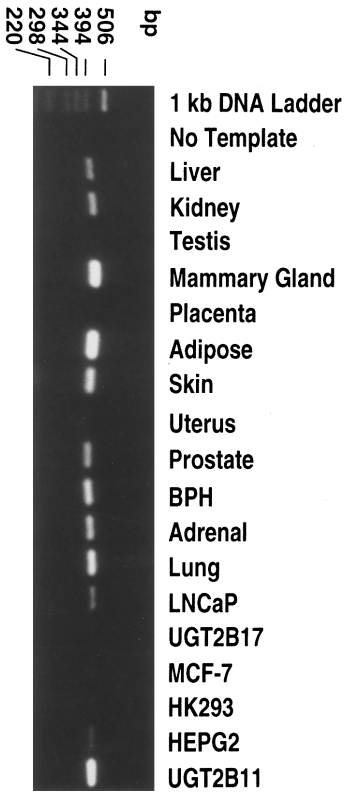

FIG. 3. Tissue distribution of UGT2B11 transcripts. Total RNA isolated from (left to right) human liver, kidney, testis, mammary gland, placenta, adipose, skin, uterus, prostate, benign prostate hyperplasia (BPH), adrenal, lung, LNCaP, HK293-UGT2B17 stable cell line, MCF-7, HK293, HEPG2 and HK293-UGT2B11 stable cell line were analyzed by RT-PCR using oligonucleotides specific for UGT2B11. One fifth of each PCR reaction was applied onto a 1% agarose ethidium bromide stained gel. The identity of the 408 bp PCR product was confirmed by direct sequencing.

In summary, the large distribution of UGT2B11 suggests a functional role for this protein. It is conceivable that UGT2B11 may constitute an orphan enzyme with undefined substrates, or it could interact with other UGT enzymes generating differential enzymatic complexes. The expression of highly homologous UGT proteins with unknown transferase activity, UGT2B10 and UGT2B11, raises the possibility that these two UGT proteins might share similar functions. The elucidation of the biological relevance of the UGT2B11 protein will require further investigations.

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