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# Use of Cloned and Expressed Human Liver UDP-glucuronosyltransferases for Analysis of Drug Glucuronide Formation and Assessment of Drug Toxicity

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Five cloned human hepatic UDP-glucuronosyltransferase (UGT) cDNAs were stably expressed in tissue culture cell lines. More than 100 drugs and xenobiotics were used as substrates for glucuronidation catalyzed by the cloned human transferases to determine the chemical structures accepted as substrates. UGT-HP1 exhibited a limited substrate specificity for planar phenolic compounds, whereas UGT-HP4 was more accepting of nonplanar phenols, anthraquinones, flavones, aliphatic alcohols, aromatic carboxylic acids, steroids and many drugs of varied structure. UGT-HP3 (bilirubin UGT) catalyzed the glucuronidation of ethinylestradiol. UGT-H6 and UGT-H25 (steroid/bile acid UGTs) also catalyzed the glucuronidation of some xenobiotics. Levels of UGT-HP4 activity towards some substrates were sufficient to allow determination of kinetic parameters for the enzyme reaction. Further, metabolism of drugs could be studied by addition to the recombinant cell lines in culture and extraction of the media allowed analysis of glucuronide formation. The protection afforded against cytotoxic drugs was observed. The data presented here demonstrate the potential of using these recombinant cell lines for investigation of phase II metabolism by human UGTs and subtle differences in protein structure which affect their specificity. —*Environ Health Perspect* 102(Suppl 9):19–23 (1994)

Key words: stable expression, phenols, steroids, bilirubin, multigene family

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## Introduction

Numerous drugs and xenobiotics are excreted as glucuronide conjugates. For many of these compounds, glucuronidation is the major route of detoxication and safe elimination (1,2).

The important feature of human glucuronidation is that identical systems may not exist in common laboratory species, such as the ability to form quaternary ammonium glucuronides of tertiary amine drugs (2). Inaccessibility to humans and their tissues for experimentation has severely restricted studies of drug glucuronidation systems and the use of this knowledge for drug discovery.

Therefore, alternative systems, which attempt to mimic human glucuronidation need to be devised. We have developed the use of recombinant cell lines expressing human UGTs to facilitate the study of the biochemistry of glucuronidation in physiological systems. These systems have great potential for our understanding of the specificity of human glucuronidation, for drug development and prediction of toxicity.

### The UGT Superfamily of Genes and Enzymes

The UGTs are a group of isoenzymes of 50–60 kDa localized primarily in hepatic endoplasmic reticulum and nuclear envelope (3). The UGT superfamily (4) has evolved to catalyze the glucuronidation of potentially toxic endobiotics and ancient environmental xenobiotics, thereby facilitating transport and excretion of more water-soluble glucuronides. The glucuronidation of drugs by this system is probably fortuitous as the pace of the chemical revolution has easily overtaken biological evolution. The expression of UGTs is differentially regulated by xenobiotic and hormonal inducers and during development, such that different tissue specific profiles of activities are observed (5).

A nomenclature for the UDP-glucuronosyltransferase superfamily has been proposed, based on divergent evolution of the genes (4). A total of 26 distinct cDNAs in five mammalian species have been cloned and sequenced to date. Comparison of deduced amino acid sequences has led to the definition of two families and a total of three subfamilies.

Nine human liver UGT cDNAs have recently been cloned and two subfamilies have been classified by sequence identities (6). Subfamily 1 consists of at least four UGTs that catalyze the glucuronidation of xenobiotic phenols and bilirubin (7,8), but not some steroids or bile acids. Interestingly, these four cDNAs encoding UGTs share identical C-terminal 246 amino acid residue sequences, but variable N-terminal 285 amino acid residue sequences.

Construction of hybrid proteins (9) and comparative sequence analysis of UGTs (5) suggest the substrate binding specificity is provided by the N-terminal half of proteins and that UDP-glucuronic acid binds in the C-terminal portion probably involving key amino acids between residues 350 and 400.

Evidence from Southern-blot analysis indicates that there is only one copy of the conserved 3' region of the UGT1 gene in

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the human genome. Chromosomal mapping of the sequences encoding the four variable regions using specific probes indicated that all of these sequences were located on human chromosome 2 (10,11). These results suggest that at least four isoenzymes may be derived from variable exons and conserved exons within the same gene. The above studies have been authenticated by the work of Ritter et al. (12). They have reported the isolation of four human cosmid clones that span 110 kb and contain the four constant 3' exons on the phenol/bilirubin UGTs. Moreover these genomic clones contain six variable 5' exons that include the coding sequence for HP1, HUGBr1, and HUGBr2 as well as three other UGT coding exons. It has been proposed that these UGT mRNAs result from alternative splicing from this gene complex. Subfamily 2 contains at least five UGTs catalyzing steroid or bile acid glucuronidation (13–15). Subfamily 2 appears to consist of several separate genes each encoding a unique steroid UGT while retaining a similar gene structure even in different species (16,17). These genes appear to be localized in a cluster on chromosome 4 (17).

The analysis of the structural features of UGTs and the proteolytic/immunochemical analysis of proteins in the ER membrane (18,19) has provided insights into the transverse topology of these enzymes. These studies suggest that the major portion of the proteins (including the active sites) reside within the lumen of the ER membrane and that the protein is retained in this organelle by a C-terminal membrane spanning region and a charged cytosolic retention signal peptide (3).

The proteins are synthesized and inserted into endoplasmic reticulum using a series of signal sequences. An N-terminal signal peptide (approximately 23AA residues), which is subsequently removed (3), targets the protein into the ER membrane; charged C-terminal lysines and a membrane spanning region establish retention and orientation in the membrane (20,21). This general structure may be further stabilized by the known glycosylation of the UGTs (3).

This UGT localization in microsomal membranes embraces the concept of latent enzyme activities. Microsomal UGTs are activated up to 20-fold by detergent disruption or proposed physiological activators such as UDP-*N*-acetylglucosamine (22). The luminal compartmentalization of UGT activities within the ER creates a barrier for substrate and UDP-glucuronic

acid access to the active site invoking a possible need for transporters assisting in glucuronidation (23).

Therefore, the use of recombinant cell lines expressing UGTs to duplicate and facilitate understanding of the physiological systems should consider orientation of UGTs into ER. Fortunately, biological evolution has allowed development of both consistent and versatile mechanisms, which facilitate experimental creation of artificial but functional cell systems.

Here we describe how cloned expressed UGT isoenzymes have been used to study drug glucuronidation and the potential role of glucuronidation in protection of cells against cytotoxic agents.

## Methods

### Cloning and Stable Transfection of UGTs into Tissue Culture Cells

Human liver cDNA libraries in various bacteriophage expression vectors were screened with several nucleic acid probes. The protocols used to screen the libraries and obtain full length clones, have been described in detail elsewhere (6,7).

The UGT cDNAs were subcloned into the eukaryotic expression vector pcDNAneo. Linearized recombinant plasmid DNA was introduced into Chinese hamster V79 lung fibroblasts facilitated by the transfection reagent DOTAP. Stably transfected colonies (G418-resistant) were isolated and cultured as previously described (24).

### Analysis of Tissue Culture Cell Homogenates and Human Liver Microsomes

UGT-V79 cells were washed twice with PBS and gently homogenized (24). Human liver microsomal fractions were prepared as described (25). SDS polyacrylamide gel electrophoresis and Western blotting of homogenates and microsomes were performed as previously described (7).

Assay of UGT activities in cell homogenates was determined by TLC assay using 0.5 mM substrate and 2 mM UDPGA (including [<sup>14</sup>C] 2μCi labeled UDPGA) (26). Bilirubin UGT activity was determined as previously described (27). Protein levels were assayed by the Lowry method (28).

### Chemosensitivity Testing

UGT-recombinant cell lines were seeded into 24 well plates at 1000 cells/well to allow exponential growth during the course of the assay (4 days). Cells were grown in DMEM supplemented with 10% fetal calf

serum, 2 mM glutamine and 100 U/ml penicillin/streptomycin and maintained in a humidified atmosphere of 5% CO<sub>2</sub>–95% air at 37°C. Cytotoxic drugs were added in a range of concentrations from 10 × stock solutions to cells for 2 days. A viable cell count was then performed using the MTT assay (29).

## Results and Discussion

### Expression of Human Family UGTs in V79 Cell Lines

Immunochemical analysis of homogenates of cell lines showed the presence of high levels of expressed enzymes UGT1 subfamily, UGT-HP1 (Mr 55 kDa), UGT-HP3 (Mr 55.5 kDa), and UGT-HP4 (Mr 56 kDa) (6,30). Further immunochemical analysis of whole cells has revealed the localization of the enzymes in the endoplasmic reticulum and nuclear envelope (24). The UGT1A1 (HP1) expressed in cell cultures has been demonstrated to be a latent glycosylated enzyme suggesting the correct insertion and orientation of UGT in the microsomal membranes (Pritchard, Fournel-Gigleux, Bock and Burchell, unpublished data).

### Drug and Xenobiotic Glucuronidation by Cloned Expressed Human UGT1 Isoenzymes

This gene subfamily has at least four known expressed isoenzymes derived from the same gene (12), where cloned cDNAs have been used to study the substrate specificity of the isoenzymes. Two of these isoenzymes (HP2 and HP3) appear to be specifically responsible for catalyzing bilirubin glucuronidation (8,30), and UGT-HP1, and UGT-HP4 catalyze glucuronidation of simple or bulky phenols respectively (7,26,31). The substrate specificity of these enzymes is now being extensively studied and all the isoenzymes are able to catalyze the glucuronidation of some xenobiotic chemicals. A selection of the results from our published work are summarized in Table 1.

The difference between the two expressed phenol UGTs (HP1 and HP4) is their ability to glucuronidate phenols substituted with increasingly bulky alkyl groups. The transferase encoded by UGT-HP1 exhibits a high activity towards planar phenols such as 1-naphthol, 4-methylphenol, and 4-ethylphenol. However, expressed UGT-HP4 shows a high activity towards 4-propylphenols, 4-butylphenols and pentylphenols, and 4-hydroxybiphenyl; whereas little or no activity was

**Table 1.** Substrate specificity of stably expressed human UGT1 isoenzymes.

Substrate	UGT isoenzyme, nmole/min/mg/protein		
	HP1	HP3	HP4
1-Naphthol	3.2	0.3	0.2
Vanillin	2.4	–	0.9
Ergenol	1.1	–	1.2
4-Ethylphenol	1.3	–	1.9
4- <i>tert</i> -Butylphenol	0.2	–	3.1
5- <i>n</i> -Octylgallate	0.1	–	5.3
Emodin	0.0	–	1.9
Quercetin	0.0	–	1.8
Propofol	ND	ND	1.2
Labetol	–	–	0.06
Propranolol	–	–	0.04
Diflunisal	–	–	0.05
Ethinylestradiol	ND	0.35	0.14
4-Hydroxyestrone	–	–	0.45
Ibuprofen	–	–	0.08
Methylethyl hexyl phthalate	ND	ND	0.17
Retinoic acid	–	–	0.05
Phenolphthalein	–	–	0.47
Bilirubin	ND	0.40	ND
Triphenylheptanoic acid	ND	0.06	ND

Abbreviations –, not assayed; ND, not detected.

detected using UGT-HP1. This remarkable difference suggests that structural differences within the active sites of the two enzymes limit the dimensions of substrate accepted by UGT-HP1. This transferase will accept benzo[*a*]pyrene-3, 6-quinol and performed better with 1-naphthol as substrate (32), and therefore the planarity of the substrate accepted seems to be a most important criterion.

However, it is apparent that two enzymes may be involved in the metabolism of a single substrate as demonstrated by biphasic kinetics of microsomal glucuronidation. Their individual performance may be dependent on the concentration of the aglycone. Paracetamol is glucuronidated by both UGTs HP1 and HP4; but HP1 has an apparent  $K_m$  of 2.5 mM, whereas HP4 exhibited an apparent  $K_m$  of 50 mM (33). The results would suggest that UGT-HP1 is the high affinity paracetamol glucuronidating enzyme in man.

Our work has demonstrated that UGT-HP4 is capable of catalyzing the glucuronidation of a wider range of compounds in terms of the general structure and the functional group which is glucuronidated. High levels of transferase activity for compounds with phenolic-OH groups such as the anaesthetic propofol (2,6-diisopropyl phenol) and also several phenols of plant origin such as thymol, carvacrol, and eugenol were observed. The latter two compounds are also glucuronidated by the

UGT-HP1 isoenzyme whereas only minute amounts of the glucuronides of thymol and no glucuronides of propofol were formed by UGT-HP1. This is probably due to the bulky isopropyl substituents in position 2 to the phenolic OH-group (thymol is 2-isopropyl-5-methylphenol). UGT-HP4 also showed activity for the glucuronidation of terpenoids with aliphatic OH groups such as carveol, citronellol, nopol and spinocapheol. Surprisingly, carboxylic acids (substituted propionic acids used as nonsteroidal antiinflammatory drugs like ibuprofen, fenoprofen, or naproxen) were also substrates for this enzyme. The ability of UGT-HP4 to glucuronidate bulkier, more complex molecules is also reflected in the formation of glucuronides of fluorescein, phenolphthalein, anthraquinones (such as emodin), and flavones (such as quercetin).

UGT-HP4 catalyzed the glucuronidation of food preservatives, e.g., esters of 4-hydroxybenzoic acid and vanillin and also *N*-glucuronidation of dapsone. This human transferase is apparently a key enzyme in the detoxication of many xenobiotic compounds and drugs (26). Neither of the cloned, expressed UGTs catalyzed the glucuronidation of (–) morphine, estriol, or androsterone, although high activity of UGT-HP4 towards 4-OH-estrone was observed. The more suitable endogenous or xenobiotic substrates may remain to be identified.

The bilirubin UGT (HP3) was a poor catalyst of most substrates tested (Table 1), although ethinylestradiol was a good substrate for this enzyme and further kinetic analysis of human liver microsomes has indicated that bilirubin UGT may be responsible for catalyzing ethinylestradiol glucuronidation in humans (26).

### Drug and Xenobiotic Glucuronidation by Cloned and Expressed UGT2 Isoenzymes

The first full-length human liver UGT cDNA (H25) was published by Jackson et al. (34). The derived protein sequence exhibits an identity of 64% to the sequence derived from the rat liver UGT cDNA Rlug38, testosterone UGT (35). Subsequently, UGT-H25 was expressed in cell cultures and determined to catalyze the glucuronidation of hyodeoxycholic acid but not xenobiotics (13,24). Recently, Ritter et al. (12) have shown that this enzyme also catalyzed the glucuronidation of estriol and 4-hydroxyestrone. Jackson screened a human liver cDNA library with UGT-H25 at low stringency. He isolated three additional UGT cDNAs, H4, H7, and H32. The identity between these human UGT cDNA clones varied from 66 to 75%. However, only UGT-H25 was full length. Therefore, another human liver cDNA library was screened with UGT-H25. This identified six putative positives, including UGT-H14 and UGT-H6.

Ritter et al. (37) published the sequence of human liver UGT cDNA, UDP-GT<sub>h2</sub>, that has an identity of 96% when compared to UGT-H6. The high identity between UGT-H6 and UDP-GT<sub>h2</sub> indicates that these two cDNAs may be derived from different alleles of the same gene.

When expressed in cell cultures both of these enzymes primarily catalyze the glucuronidation of estrogens. Ritter et al. (37) tested this transferase with some 20 xenobiotic substrates and no detectable glucuronides were formed. Although another very similar expressed cloned UGT has been shown to slowly catalyze the glucuronidation of carboxylic acid drugs such as clofibrate, valproate, naproxen, fenoprofen, and zomepirac, although quantitative data have not yet been reported (38).

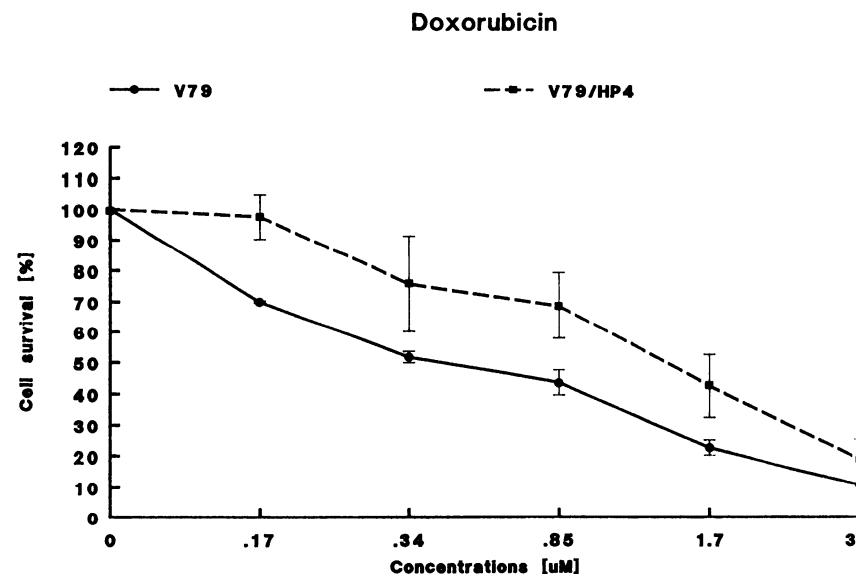
Burchell et al. (4) have proposed that any two UGT cDNAs with less than 97% identity are derived from different genes. Nebert and Gonzalez (39) arbitrarily assign P450 cDNAs as being derived from different mRNAs and different genes when their identity is >97% but where functional dif-

ferences have been shown. Nebert and Gonzalez (39) do not allow for cDNAs that have identities of <97% that show functional similarities. Although UGT-H6 and UDP-GT<sub>h2</sub> do have similar catalytic properties, the coding sequence for UDP-GT<sub>h2</sub> is six bases longer and 50 other base changes were observed, introducing some charge differences to the sequence (40).

With these base changes and the cut-off point for the P450 genes, it is proposed that UGT-H6 and UDP-GT<sub>h2</sub> are derived from different genes. This proposal will only be proven by the isolation of further human UGT cDNAs or from the isolation of the genes encoding these isoforms.

### Assessment of Resistance to Xenobiotic and Anticancer Drug-induced Toxicity in Recombinant Cell Lines Stably Expressing Human UGTs

In a preliminary experiment, UGT-HP1 stably expressed in NIH-3T3 cells was shown to enhance cell survival 10-fold against mitoxanthrone, when compared to nontransfected 3T3 cells (41). Similar results have been demonstrated using other expressed UGTs and cytotoxic drugs. Figure 1 shows that stably expressed UGT-HP4 (a phenol UGT) enhanced cell survival 5- to 10-fold against doxorubicin. The additional protection against these cytotoxic anticancer drugs is presumably caused by an increased rate of glucuronidation and facilitated excretion of these compounds. Indeed, doxorubicin was also shown to be glucuronidated by human



**Figure 1.** Cellular protection against doxorubicin toxicity is increased by an expressed human liver UGT-HP4. Chinese hamster lung fibroblast V79 cells and V79 cells stably transfected with UGT-HP4 were exposed to increasing concentrations of doxorubicin. —●—, V79 cells; —■—, V79/HP4 cells.

liver microsomal preparations (unpublished data).

### Conclusions

Clearly there is enormous potential of cloned human UGTs expressed in tissue culture cells in the study of drug glucuronidation. Several cloned transferases stably expressed in cell lines are already available. Additional cloned UGTs will become available in the near future.

Therefore, we should be in a position to examine the abilities of these cloned transferases to catalyze the glucuronidation of therapeutically important drugs and assess the potential dangers of polymorphic variation of UGTs in routine drug treatments. Further, the important role of UGTs in cellular defense against cytotoxic chemicals has been clearly demonstrated, indicating the value of these cell lines in defining this role.

### REFERENCES

- Mulder GJ, Coughtrie MWH, Burchell B. Glucuronidation. In: *Conjugation Reactions in Drug Metabolism* (Mulder GJ, ed). London: Taylor and Francis, 1990;51-105.
- Coughtrie MWH. Role of molecular biology in the structural and functional characterisation of the UDP-glucuronosyltransferases. *Prog Drug Metab* 13:36-68 (1991).
- Tephly TR, Burchell B. UDP-glucuronosyltransferases: a family of detoxifying enzymes. *TIPS* 11:276-280 (1990).
- Burchell B, Nebert DW, Nelson DR, Bock KW, Takashi I, Jansen PLM, Lancelot D, Mulder GJ, Chowdhury JR, Siest G, Tephly TR, Mackenzie PI. The UDP glucuronosyltransferase gene superfamily: suggested nomenclature based on evolutionary divergence. *DNA Cell Biol* 10:487-494 (1991).
- Burchell B, Coughtrie MWH. UDP-glucuronosyltransferases. *Pharmacol Ther* 43:261-289 (1989).
- Sutherland L, Wooster R, Jackson MR, Harding D, Fournel-Gigleux S, Burchell B. Cloning and expression of UDP-glucuronosyltransferase cDNAs. In: *Hepatic Metabolism and Disposition of Endo- and Xenobiotics* (Bock KW, Gerok W, Matern S, eds). Lancaster, UK: Kluwer Academic Publishers, 57:141-150 (1991).
- Wooster R, Sutherland L, Ebner T, Clarke D, De Cruz E, Silva O, Burchell B. Cloning and stable expression of a new member of the human phenol/bilirubin UDP-glucuronosyltransferase cDNA family. *Biochem J* 278:465-469 (1991).
- Ritter JK, Crawford JM, Owens IS. Cloning of two human liver bilirubin UDP-glucuronosyltransferase cDNAs with expression in COS-1 cells. *J Biol Chem* 266:1043-1047 (1991).
- Mackenzie PI. Expression of Chimeric cDNAs in cell culture defines a region of UDP-glycyronosyltransferase involved in substrate selection. *J Biol Chem* 265:3432-3435 (1990).
- Moghrabi N, Sutherland L, Wooster R, Povey S, Boxer M, Burchell B. Chromosomal assignment of human phenol and bilirubin UDP-glucuronosyltransferase genes (UGT1A-subfamily). *Ann Hum Genet* 56:83-93 (1992).
- Harding D, Jeremiah SJ, Povey S, Burchell B. Chromosomal mapping of a human phenol UDP-glucuronosyltransferase, GT1. *Ann Hum Genet* 54:17-21 (1990).
- Ritter JK, Chen F, Sheen YY, Tran HM, Kimura S, Yeatman MT, Owens IS. A novel complex locus UGT1 encodes human bilirubin, phenol and other UDP-glucuronosyltransferase isozymes with identical carboxy termini. *J Biol Chem* 267:3257-3261 (1992).
- Fournel-Gigleux S, Jackson MR, Wooster R, Burchell B. Expression of a human liver cDNA encoding UDP-glucuronosyltransferase catalysing the glucuronidation of hydoxycholeic acid in cell culture. *FEBS Lett* 243:119-122 (1989).

14. Coffman BL, Tephly TR, Irshaid YM, Green MD, Smith C, Jackson MR, Wooster R, Burchell B. Characterisation and primary sequence of human hepatic microsomal estriol UDP-glucuronosyltransferase. *Arch Biochem Biophys* 281:170-175 (1990).
15. Ritter JK, Sheen YY, Owens IS. Cloning and expression of human liver UDP-glucuronosyltransferases in COS-1 cells. *J Biol Chem* 266:7900-7906 (1990).
16. Mackenzie PI, Rodbourn L. Organisation of the rat UDP glucuronosyltransferase, UDPGTr-2 gene and characterisation of its promoter. *J Biol Chem* 265:11328-11333 (1990).
17. Monaghan G, Povey S, Burchell B, Boxer M. Localization of a bile acid UDP-glucuronosyltransferase gene to chromosome 4 using the polymerase chain reaction. *Genomics* 13:908-909 (1992).
18. Vanstapel F, Blanckaert N. Topology and regulation of bilirubin UDP-glucuronosyltransferase in sealed native microsomes from rat liver. *Arch Biochem Biophys* 263:216-225 (1988).
19. Shepherd RP, Baird SJ, Hallinan T, Burchell B. An investigation of the transverse topology of bilirubin UDP-glucuronosyltransferase in rat hepatic endoplasmic reticulum. *Biochem J* 259:617-620 (1989).
20. Nilsson T, Jackson MR, Peterson PA. Short cytoplasmic sequences serve as retention signals for transmembrane proteins in the endoplasmic reticulum. *Cell* 58:707-718 (1989).
21. Jackson MR, Nilsson T, Peterson PA. Identification of a consensus motif for retention of transmembrane proteins in the endoplasmic reticulum. *EMBO J* 9:3153-3162 (1990).
22. Dutton GJ, Burchell B. Newer aspects of glucuronidation. *Prog Drug Metab* 2:1-70 (1977).
23. Burchell B, Burchell A. Molecular pathologies of the endoplasmic reticulum. *Curr Opin Cell Biol* 1:712-717 (1989).
24. Fournel-Gigleux S, Sutherland L, Sabolovic N, Burchell B, Siest G. Stable expression of two human liver glucuronosyltransferases in V79 cell cultures. *Mol Pharmacol* 39:177-183 (1991).
25. Coughtrie MWH, Blair JNR, Hume R, Burchell A. Improved procedure for the preparation of hepatic microsomes to be used in the *in vitro* diagnosis of inherited disorders of the glucose-6-phosphatase system. *Clin Chem* 37:739-742 (1991).
26. Ebner T, Burchell B. Substrate specificities of two stably expressed human liver UDP-glucuronosyltransferases of the UGT1 gene family. *Drug Metab Disp* 21:50-55 (1992).
27. Heirwegh KPM, Van de Vijver M, Fevery J. Assay and properties of digitonin-activated bilirubin uridine diphosphate glucuronosyltransferase from rat liver. *Biochem J* 129:605-618 (1972).
28. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin-phenol reagent. *J Biol Chem* 193:265-275 (1951).
29. Carmichael J, De Graff WG, Gazdar, AF, Minna JD, Mitchell JB. Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of chemosensitivity testing. *Cancer Res* 47:936-942 (1987).
30. Sutherland L, Senafi S, Ebner T, Clarke DJ, Burchell B. Characterisation of a human bilirubin UDP-glucuronosyltransferase stably expressed in hamster lung fibroblast cell cultures. *FEBS Lett* 308:161-164 (1992).
31. Harding D, Fournel-Gigleux S, Jackson MR, Burchell B. Cloning and substrate specificity of a human phenol UDP-glucuronosyltransferase expressed in COS-7 cells. *Proc Natl Acad Sci USA* 85:8381-8385 (1988).
32. Bock KW, Gschaidmeier H, Seidel A, Baird S, Burchell B. Mono- and diglucuronide formation of chrysene and benzo(a)pyrene phenols by 3-methylcholanthrene-inducible phenol UDP-glucuronosyltransferase (UGT1A1). *Mol Pharmacol* 42:613-618 (1992).
33. Bock KW, Forster A, Gschaidmeier H, Bruck M, Munzel P, Schareck W, Fournel-Gigleux S, Burchell B. Paracetamol glucuronidation by recombinant rat and human phenol UDP-glucuronosyltransferases. *Biochem Pharmacol* 45:1809-1814 (1993).
34. Jackson MR, McCarthy LR, Harding D, Wilson S, Coughtrie MWH, Burchell B. Cloning of a human liver microsomal UDP-glucuronosyltransferase cDNA. *Biochem J* 242:581-588 (1987).
35. Harding D, Wilson SM, Jackson MR, Burchell B, Green MD, Tephly TR. Nucleotide and reduced amino acid sequence of rat liver 17 $\beta$  hydroxysteroid UDP-glucuronosyltransferase. *Nucleic Acids Res* 15:3936 (1987).
36. Harding D, Jackson MR, Wooster R, Fournel-Gigleux S, Burchell B. Cloning of human UDP-glucuronosyltransferase cDNAs. In: *Cellular and Molecular Aspects of Glucuronidation* (Siest G, Magdalou J, Burchell B, eds). Montrouge, France: John Libbey Eurotext/INSERM, 1988;43-50.
37. Ritter JK, Sheen YY, Owens IS. Cloning and expression of human liver UDP-glucuronosyltransferase in COS-1 cells. *J Biol Chem* 265:7900-7906 (1990).
38. Jansen PLM, Mulder GJ, Burchell B, and Bock KW. New developments in glucuronidation research. Report of a Workshop on Glucuronidation, Its Role in Health and Disease. *Hepatology* 15:532-544 (1992).
39. Nebert DW, Gonzalez F. P450 genes: structure, evolution and regulation. *Ann Rev Biochem* 56:943-993 (1987).
40. Wooster RW. The molecular cloning of human UDP-glucuronosyltransferase cDNAs and their expression in tissue culture cells. PhD Thesis, University of Dundee, Scotland (1991).
41. Burchell B, Baird S, Coughtrie MWH. The role of xenobiotic glucuronidating enzymes in drug resistance of tumour tissues and cells. In: *Xenobiotics and Cancer* (Ernster L, Esumi H, Fujii Y, Gelboin HV, Kato R, Sugimura T, eds) London: Taylor & Francis, 1991;263-275.