Cloning of UGT1A9 cDNA from liver tissues and its expression in CHL cells

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Abstract

AIM: To clone the cDNA of UGT1A9 from a Chinese human liver and establish the Chinese hamster lung (CHL) cell line expressing human UGT1A9.

METHODS: cDNA of UGT1A9 was transcribed from mRNA by reverse transcriptase-polymerase chain reaction, and was cloned into the pGEM-T vector which was amplified in the host bacteric E. Coli DH5α. The inserted fragment, verified by DNA sequencing, was subcloned into the HindIII/NotI site of a mammalian expression vector pREP9 to construct the plasmid termed pREP9-UGT1A9. CHL cells were transfected with the resultant recombinants, pREP9-UGT1A9, and selected by G418 (400 μg ml⁻¹) for one mo nth. The surviving clone (CHL-UGT1A9) was harvested as a pool and sub-cultured in medium containing G418 to obtain samples for UGT1A9 assays. The enzyme activity of CHL-UGT1A9 towards propranolol in S9 protein of the cell was determined by HPLC.

RESULTS: The sequence of the cDNA segment cloned, which was 1666 bp in length, was identical to that released by Gene Bank (GenBank accession number: AF056188) in coding region. The recombinant constructed, pREP9-UGT1A9, contains the entire coding region, along with 18 bp of the 5’ and 55 bp of the 3’ untranslated region of the UGT1A9 cDNA, respectively. The cell lines established expressed the pro tein of UGT1A9, and the enzyme activity towards propranolol in S9 protein was found to be 101±24 pmol·min⁻¹·mg⁻¹ protein (n = 3), but was Not detectable in parental CHL cells.

CONCLUSION: The cDNA of UGT1A9 was successfully cloned from a Chinese human liver and transfected into CHL cells. The CHL-UGT1A9 cell lines established efficiently expressed the protein of UGT1A9 for the further enzyme study of drug glucuronidation.

Subject headings UGT1A9; cloning; glucuronidation; cell lines

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INTRODUCTION

Most organisms are exposed to a range of lipophilic compounds and converted them into excretable hydrophilic compounds. This metabolism of foreign compounds (xenobiotics) can be divided into two phases. For phase I metabolism, a reactive group is mostly introduced into the xenobiotic molecule. These reactions are mainly catalyzed by the cytochrome P450 monoxygenase system which consists of cytochrome P450s (CYPs) and cytochrome P450 reductase (CPR). For phase II metabolism, the reactive metabolite is conjugated to small, hydrophilic endogenous molecules such as glucuronic acid. The conjugation of this cofactor to xenobiotics is catalyzed by UDP-glucuronosyltransferases (UGTs). Since xenobiotic metabolizing enzymes have to catalyze the metabolism of structurally very divers e substrates, the various enzyme systems (e.g. CYPs and UGTs) comprise several isozymes that differ in their catalytic properties. The members of a given enzyme system have been grouped into families and subfamilies based on sequence homologies. In UGTs, two enzyme families termed UGT1 and UGT2 have been described. The UGT1 locus is highly conserved between species[1]. UGT1A is a subfamily of UGT1 gene complex that is located at chromosome 2q37. UGT1A subfamily is encoded by tandem individual promoters and their first exons are linked by differential splicing to four common exons. As one of the isoforms, UGT1A9, is mainly expressed in liver. UGT1A9 can be induced by polycyclic aromatic hydrocarbons (PAHs), and therefore the drug glucuronidation catalyzed by UGT1A9 will be increased in cigarette smokers who inhale PAHs[2].

Human hepatic UDP-glucuronosyltransferases (UGT) is a family of microsomal enzymes that catalyze the glucuronidation of many important drugs, xenobiotics and endogenous compounds. Attempts to characterize the microsomal enzymes by conventional purification technique are often frustrated due to its instability. UGT isoenzyme expressed by cells is a useful tool for characterizing UGT’s function. The cDNA cloning of UGTs from various sources (rabbit, rat, monkey, human beings, etc.) and their expression in cell lines were widely used for the gene characterization and function study of UGT isoforms[3-9]. In order to study drug metabolisms by UGTs, the cDNA encoding UGT1A9 was cloned from human liver and expressed in Chinese hamster lung (CHL) cell line in this study. The enzyme expressed was extracted and its activity was assayed with a substrate of propranolol which is a nonselective β-adrenergic blocking agent and can be used widely clinically[10].

MATERIALS AND METHODS

Isolation of RNA from human liver tissue

Human liver tissue was obtained from a surgical specimen of Chinese and stored at -80°C until use. The total RNA was isolated with TRIzol reagent (Gibco Corp, USA)

UGT1A9 cDNA transcription

cDNA was transcribed from mRNA by reverse transcriptase polymerase chain reaction (RT-PCR). Five μg of the total RNA and 2 μg of random primer (SANGON, Shanghai) in deionized water containing
An agarose gel electrophoresis was carried out with 10 µL. The mixtures were pre-incubated and the glucuronidation of 100 µmol·L⁻¹ propranolol, 1 mmol·L⁻¹ UDPGA, 1 g·L⁻¹ Triton X-100, 50 µg of S9 protein in 50 mmol·L⁻¹ Tris-HCl, 10 mmol·L⁻¹ MgCl₂ buffer, pH 7.8 at 37°C. The mixtures were pre-incubated and the glucuronidation was started by the addition of UDPGA and stopped after 2 h by the addition of 100 µL of methanol. The mixtures were stirred thoroughly and centrifuged at 10 000 r·min⁻¹ for 10 min. Un-reacted propranolol in the layer of reactant was determined by HPLC and the enzyme activity was calculated according to the amount of propranolol declined after incubation.

**HPLC analysis of propranolol metabolized by S9 of CHL-UGT1A9**

The concentration of propranolol metabolized by S9 of CHL-UGT1A9 was assayed by the HPLC procedure[11] with modification to the mobile phase. Twenty mL of the sample was applied to a reversed phase column (Shim-pack CLC-ODS15 cm×0.6 cm id, 10 µm particle size). Propranolol was monitored with a UV detector at 290 nm. The mobile phase is made up with ammonium acetate buffer (4.0 g ammonium acetate, 10 mL acetate acid and de-ionized water in 1 L)-methanol-acetonitrile (2:1:1), and to 500 mL mobile phase add 0.7 mL ammonium acetate, 10 mL acetate acid and de-ionized water in 1 L. The mobile phase is made up with ammonium acetate buffer (4.0 g ammonium acetate, 10 mL acetate acid and de-ionized water in 1 L) then the supernatant (S9) was transferred carefully to a clean tube for assay or storage under -70°C. The protein in S9 was determined by the same method that was used in our previous paper[11].

**RESULTS**

**Construction of recombinants**

The recombinant of pGEM-UGT1A9 (Figure 1) was constructed with the human UGT1A9 inserted into the cloning site of vector pGEM- T between the promoters of T7 and SP6. Selection and identification of the recombinant was carried out by *Hind III* and *Not I* endonuclease digestion and agarose electrophoresis (Figure 3). The cloned DNA segments in selected recombinants were sequenced completely. According to the results of DNA sequencing, the cDNA in a selected recombinant was identical to the DNA sequence of UGT1A9 reported by Ciotti-M et al[12] (GenBank accession no. AF056188) in the reading frame.

**Figure 1** Scheme of pGEM-UGT1A9.
The Hind III/Not I fragment (1.5 kb) containing the complete UGT1A9 cDNA was subcloned into the Hind III/Not I site of mammalian expression vector pRE P9 (Figure 2). Selection and identification of the recombinants were carried out by Hind III/Not I endonuclease digestion and agarose electrophoresis (Figure 3). The resulting plasmid was designated as pREP9-UGT1A9 which contained the entire coding region, along with 18 bp of the 5' and 55 bp of the 3' untranslated region of the UGT1A9 cDNA, respectively. In addition, the neo gene of the plasmid confers the G418 resistant phenotype to CHL cells for the selection of transfected cells.

**Establishment of recombinant cell lines with UGT1A9 enzyme activity**

CHL cells were transfected with pREP9-UGT1A9, and selected with G418 (400 mg·L⁻¹). The surviving clone was propagated and the cell line termed CHL-UGT1A9 was established. The preparation S9 was prepared from CHL-UGT1A9 cells harvested for UGT1A9 activity assay by HPLC. Figure 4 shows the typical elution of propranolol in incubation solution. The UGT enzyme activity towards propranolol in S9 protein was found to be 101±24 pmol·min⁻¹·mg⁻¹ (n = 3), but was not detectable in parental CHL cells.

**DISCUSSION**

UGTs are involved in the conjugation of UDP-glucuronic acid (UDPGA) to a variety of chemicals, drugs, and endogenous compounds. The elimination of hydrophobic chemicals from cells is aided by their conversion to water-soluble glucuronides. UGTs are closely related to the system of cytochrome P450 monooxygenase, and involved in the transportation of carrier and the passage of drugs through cell phospholipid bilayer. In most cases, the lipophilic compounds are converted by phase I metabolism to the substrate for glucuronidation by obtaining an essential function (such as carbon, nitrogen, sulfur and oxygen), but in many cases xenobiotics and endogenous substances can also be glucuronidated by UGTs without the phase I metabolism. The xenobiotic metabolizing cytochrome P450 monooxygenase system and the UGTs reside mainly in the endoplasmic reticulum. However, CYPs and the CPR are localized on the cytosolic side of the endoplasmic reticulum, which the UGTs are localized on its luminal side[14]. UGTs are latent enzymes, needing activation (in general by detergents) to express its maximal activity.

Numerous reports revealed that glucuronidation is a major pathway involved in the metabolism of drugs, exogenous and numerous endogenous compounds such as bile acids and steroid hormones. Each UGTs family or subfamily has its own substrates but the substrate spectrum are partly overlapped. UGT1 has substrates such as thyroid hormone[15], SN-38[16], bilirubin[17,18], opioids, bile acids, fatty acids, retinoids, ciprofibrate, furosemide, dilunisa, catechol estrogens, coumarins, flavonoids, anthraquinones, EM-652 (an active antiestrogen)[19] and phenolic compounds[20]. UGT2 catalyzes substrates such as estrogens, androgens, morphine, AZT, and retinoic acid, epirubicin[16,21,22,23], etc. UGT1A9 is a member of UGT1A subfamily. The endogenous substrates for UGT1A9 are estrone, 4-hydroxyestrone, ethinylestradiol, retinoic acids, and exogenous substrates include propofol, propranolol, paracetamol, S-naproxen, ketoprofen, ibuprofen, entacapone, some mutagenic amines, etc[2,24-26]. UGT1A9 was found to have regioslectivity on the glucuronidation of hydroxyl group of carbohydrate-containing drugs[27].

UGTs are expressed extensively in organs and tissues, and they may play a key role in the regulation of the level and action of steroid hormones in steroid target tissues. Organs that express UGTs include liver, kidney, gastrointestinal tract[28-30], olfactory[31], jejunum,
ileum[31], prostate[32-34], colon[34]. UGT1A9 is mainly expressed in liver, and also expressed in steroid targets[35] and colon[36].

UGTs are inducible enzymes. In most cases this induction is due to increased transcription of the corresponding genes but sometimes it is also due to an improved stability of proteins. The pattern of enzymes affected is dependent on the inducing agent. Usually, phenobarbital induces mainly enzymes within UGT2 family, and methylcholanthrene induces enzymes belonging to the UGT1 family[30]. Other chemicals that induce UGTs include aryl hydrocarbon receptor ligands or oltipraz[36], flavonoid chrysin[37], and t-butyldihydroquinone and 2,3,7,8-tetrachlorodibenzo-p-dioxin[36]. etc. UGT1A9 can be induced by polycyclic aromatic hydro carbons (PAHs)[37]. On the other hand, UGTs can also be inhibited, for example by uridine diphosphateglycosyltransferase[40] and N-glycosylation is involved in the functional properties of UDP-glucuronosyltransferase enzymes[40].

To clone and express UGTs in cells can help screen substrates that is an isoform is responsible. The production of a UGT enzyme protein using transgenic cell lines is a practical manner to study its function[42-43]. We report here the cloning of UGT1A9 cDNA and establishment of a CHL cell line expressing UGT1A9 from a Chinese human liver. The full-length cDNA, UGT1A9, that encodes for a human UDP-glucuronosyltransferase protein, was isolated from a Chinese human liver total RNA. To achieve high expression levels of UGT1A9, the UGT1A9 cDNA was cloned into the eukaryotic expression vector pREP9, which we had previously used in this laboratory for the expression of human CYP450 1A1, 2B6, 3A4, etc. in CHL cells[44]. The salient feature of this vector has an EBV origin of replication and nuclear antigen (EBNA-1) to allow high-copy episomal replication in mammal cell lines. The Rous sarcoma virus long terminal repeat (RSV LTR) early promoter controls the expression of a novel human olfactory UDP-glucuronosyltransferase. The salient feature of this vector has an EBV origin of replication and nuclear antigen (EBNA-1) to allow high-copy episomal replication in mammal cell lines. The Rous sarcoma virus long terminal repeat (RSV LTR) early promoter controls the expression of a novel human olfactory UDP-glucuronosyltransferase enzyme[44].

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Conjugation with glucuronic acid is an important biotransformation pathway for a large number of clinically used drugs. In human intestinal, UGTs play an important role in the detoxification of xenobiotics compounds and, in some cases, may limit the metabolism of drugs. Conjugation with glucuronic acid is an important pathway for detoxification of many drugs. Conjugation with glucuronic acid is an important pathway for detoxification of many drugs.

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