Molecular Cloning, Expression, and Characterization of Novel Human SULT1C Sulfotransferases That Catalyze the Sulfonation of N-Hydroxy-2-acetylaminofluorene

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Upon sulfonation, carcinogenic hydroxyarylamines such as N-hydroxy-2-acetylaminofluorene (N-OH-2AAF) can be further activated to form ultimate carcinogens in vivo. Previous studies have shown that a SULT1C sulfotransferase is primarily responsible for the sulfonation of N-OH-2AAF in rat liver. In the present study, two novel human sulfotransferases shown to be members of the SULT1C sulfotransferase subfamily based on sequence analysis have been cloned, expressed, and characterized. Comparisons of the deduced amino acid sequence encoded by the human SULT1C sulfotransferase cDNA 1 reveal 63.7, 61.6, and 85.1% identity to the amino acid sequences of rat SULT1C1 sulfotransferase, mouse SULT1C1 sulfotransferase, and rabbit SULT1C sulfotransferase. In contrast, the deduced amino acid sequence of the human SULT1C sulfotransferase 2 cDNA displays 62.9, 63.1, 63.1, and 62.5% identity to the amino acid sequences of the human SULT1C sulfotransferase 1, rat SULT1C1 sulfotransferase, mouse SULT1C1 sulfotransferase, and rabbit SULT1C sulfotransferase. Recombinant human SULT1C sulfotransferases 1 and 2, expressed in Escherichia coli and purified to near electrophoretic homogeneity, were shown to cross-react with the antiserum against the rat liver SULT1C1 sulfotransferase and exhibited sulfonating activities with N-OH-2AAF as substrate. Tissue-specific expression of these novel human SULT1C sulfotransferases were examined by employing the Northern blotting technique. The results provide a foundation for the investigation into the functional relevance of these new SULT1C sulfotransferases in different human tissues/organisms.

In vertebrates, the cytosolic sulfotransferases constitute a group of enzymes that catalyze the transfer of a sulfonate group from the active sulfate, 3'-phosphoadenosine 5'-phosphosulfate, to a substrate compound containing a hydroxyl or an amino group (1–3). These enzymes are generally thought to be involved in the inactivation and/or excretion of xenobiotics or endogenous compounds such as steroid and thyroid hormones. Upon sulfonation, carcinogenic hydroxyarylamines such as N-hydroxy-2-acetylaminofluorene (N-OH-2AAF) can be further activated to form ultimate carcinogens in vivo. Previous studies have shown that a SULT1C sulfotransferase is primarily responsible for the sulfonation of N-OH-2AAF in rat liver. In the present study, two novel human sulfotransferases shown to be members of the SULT1C sulfotransferase subfamily based on sequence analysis have been cloned, expressed, and characterized. Comparisons of the deduced amino acid sequence encoded by the human SULT1C sulfotransferase cDNA 1 reveal 63.7, 61.6, and 85.1% identity to the amino acid sequences of rat SULT1C1 sulfotransferase, mouse SULT1C1 sulfotransferase, and rabbit SULT1C sulfotransferase. In contrast, the deduced amino acid sequence of the human SULT1C sulfotransferase 2 cDNA displays 62.9, 63.1, 63.1, and 62.5% identity to the amino acid sequences of the human SULT1C sulfotransferase 1, rat SULT1C1 sulfotransferase, mouse SULT1C1 sulfotransferase, and rabbit SULT1C sulfotransferase. Recombinant human SULT1C sulfotransferases 1 and 2, expressed in Escherichia coli and purified to near electrophoretic homogeneity, were shown to cross-react with the antiserum against the rat liver SULT1C1 sulfotransferase and exhibited sulfonating activities with N-OH-2AAF as substrate. Tissue-specific expression of these novel human SULT1C sulfotransferases were examined by employing the Northern blotting technique. The results provide a foundation for the investigation into the functional relevance of these new SULT1C sulfotransferases in different human tissues/organisms.

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Based on the amino acid sequences of the cytosolic sulfotransferases found in vertebrates, two gene families, the phenol sulfotransferase family (designated SULT1) and the hydroxysteroid sulfotransferase family (designated SULT2), have been classified (13, 14). The phenol sulfotransferase family presently consists of four subfamilies, phenol sulfotransferases (SULT1A), dopa/tyrosine (or thyroid hormone) sulfotransferases (SULT1B), hydroxyarlamine (or acetylaminofluorene) sulfotransferases (SULT1C), and estrogen sulfotransferases (SULT1E) (14). With the recent discovery of two new human hydroxysteroid sulfotransferases,3 the hydroxysteroid sulfotransferase family now comprises two subfamilies, SULT2A and SULT2B (cf. Fig. 3). Earlier studies have revealed the rat liver aryl sulfotransferase IV (SULT1A1) to display N-OH-2AAF sulfotransferase activity (17, 18). It was subsequently shown that the SULT1C1 sulfotransferase may be primarily responsible for the sulfonation of hydroxyarylamines in rat liver (19, 20). Interestingly, a recent study showed that, in rat liver, the primary translational products (subunits) of the dimeric SULT1A1 and SULT1C1 sulfotransferases may also interact to form a heterodimer consisting of one SULT1A1 subunit and one SULT1C1 subunit (21).

The hydroxyarlamine sulfotransferases in human tissues have been more poorly understood. A human cDNA encoding a sulfotransferase that displays 62% amino acid sequence identity to the rat liver SULT1C1 sulfotransferase has recently been reported (22). The enzymatic properties and functional
relevance of this human SULT1C sulfotransferase (designated 1), however, remain unknown. A truncated cDNA (clone ID 302867; GenBank accession number W38522) encoding likely a new human SULT1C sulfotransferase (designated 2) has also been deposited in the Expressed Sequence Tag (EST) data base (23). More recently, a nucleotide sequence corresponding to the 3′-terminal region of the open reading frame of, hypothetically, another new human SULT1C sulfotransferase (designated 3) has been detected through genomic cloning (24). No information, however, is currently available with regard to the actual expression of the mRNA encoding this new SULT1C sulfotransferase in human tissues. We report here the molecular cloning, expression, and functional characterization of two novel human SULT1C sulfotransferases (designations 1 and 2) potentially involved in the activation of carcinogenic hydroxylamines.

**EXPERIMENTAL PROCEDURES**

**Materials**—Dopamine, p-nitrophenol, aprotinin, thrombin, ATP, SDS, Hepes, Taps, Trizma (Tris base), dithiothreitol, and isopropyl-β-D-thiogalactopyranoside were products of Sigma. A chromatographically pure N-OH-2AAF was a generous gift from Dr. Danuta Malejka-Giganti at the VA Medical Center, Minneapolis, MN. Cyclist Exo-Philllarylated reverse transcription primer Sense-1

**Antisense-1**

Sense-2

**Antisense-2**

PCR primers used for for prokaryotic expression experiments

1. For full-length hSULT1C 1 sequence:

hSULT1C 1 sense

hSULT1C 1 antisense

2. For 5′ portion of hSULT1C 2 sequence:

hSULT1C 2 (5′) sense

hSULT1C 2 (5′) antisense

3. For 3′ portion of hSULT1C 2 sequence:

hSULT1C 2 (3′) sense

hSULT1C 2 (3′) antisense

**TABLE I**

Oligonucleotide primers used for PCR amplifications

| Primer Type | Sense/antisense | Nucleotide Sequence |
|-------------|-----------------|---------------------|
| 5′-Rapid amplification of cDNA ends primers | Sense-1 | 5′-pCTTCTGGTAATCTC-3′ |
| 5′-Phosphorylated reverse transcription primer | Antisense-1 | 5′-TAGAGAGAGGATAGAGATGAGGT-3′ |
| | Sense-2 | 5′-TCCCCAACTTTTACAGAT-3′ |
| | | 5′-GCCTCCTTTATCCCCATA-3′ |
| | | 5′-AAAAAGGGGGGTTAGGG-3′ |
| | | 5′-GCGGGATCCTGGCCCTGACCTCAGAATTG-3′ |
| | BamHI | 5′-GCGGGATCCTGGCCCTGACCTCAGAATTG-3′ |
| | EcoRI | 5′-ACAATTTTACTGAGACTCTGTG-3′ |
| | XbaI | 5′-CAAGTTTGAGATAATATGGTGC-3′ |
| | | 5′-CAAGTTTGAGATAATATGGTGC-3′ |
| | | 5′-GCGGGATCCTGGCCCTGACCTCAGAATTG-3′ |

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hSULT1C 2 (5′) sense

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3. For 3′ portion of hSULT1C 2 sequence:

hSULT1C 2 (3′) sense

hSULT1C 2 (3′) antisense

**DNA Library Screening of a Novel Human SULT1C Sulfotransferase**—The I.M.A.G.E. cDNA clone 302867, identified by searching through the EST data base, was subcloned into pbBlueScript II SK(+) and sequenced. Analysis of the nucleotide sequence obtained indicated that the cDNA encodes the C-terminal portion of a novel SULT1C sulfotransferase (designated 2). To prepare the probe for more efficient cDNA library screening for cDNA clones encompassing the entire open reading frame or its 5′-coding region, a 5′-rapid amplification of cDNA ends experiment was performed using the human brain total RNA as the template in conjunction with a gene-specific primer (Table I) designed based on an internal sequence of the cDNA clone number 302867. Nested polymerase chain reaction (PCR) was subsequently performed using two sets of sense and antisense primers based on the nucleotide sequence of the cDNA clone 302867 (Table I) according to manufacturer's instructions. The PCR products obtained were subcloned into pbBlueScript II SK(+) and transformed into E. coli XL1-Blue MRF+. One resulting clone (designated 5′-rapid amplification of cDNA ends-25), verified by nucleotide sequencing, was used as a probe to screen a human fetal lung 5′-stretch cDNA library. Approximately 2 × 10⁶ plaques from the library were screened. Hybridized membranes were washed once with 2 × SSC (1 × SSC, 0.15 M NaCl and 0.015 M sodium citrate) plus 0.1% SDS and twice with 0.1× SSC plus 0.1% SDS at 65 °C, followed by autoradiography to reveal the positive cDNA clones. After two additional cycles of re-screening, seven positive cDNA clones were obtained and subcloned into pbBlueScript II SK(+) and expressed in E. coli XL1-Blue MRF+. Seven deletional mutants of one of them (designated clone F-11) were prepared using Exonuclease III and mung bean nuclease (26) and subjected to nucleotide sequencing.

**DNA Sequence Determination and Analysis**—The inserts of individual pbBlueScript clones prepared as described above were sequenced in both directions using T3 or T7 sequencing primer (27). Alternatively, automated cycle sequencing was performed using Taq dimer primer cycle sequencing kits with ~21M13 or M13 reverse primer. The sequences obtained were analyzed using the E-mail server at NCBI for homology to the sequences stored in the GenBank data base.

**Expression and Purification of Recombinant Human SULT1C Sulfotransferases 1 and 2**—To amplify the human SULT1C sulfotransferase 1 sequence for subcloning into a prokaryotic expression vector pGEX-2TK, a set of sense and antisense oligonucleotide primers (Table I), based on 5′ and 3′ regions of the nucleotide sequence encoding the human SULT1C sulfotransferase 1 were synthesized with BamHI and EcoRI restriction sites incorporated at the ends. With these two oligonucleotides as primers, a PCR in a 100-μl reaction mixture was carried out under the action of Pfu DNA polymerase using the I.M.A.G.E. cDNA
clone 296479 as the template. Amplification conditions were 30 cycles of 30 s at 94 °C, 30 s at 55 °C, and 2 min at 72 °C. The final reaction mixture was applied onto a 1.2% agarose gel and separated by electrophoresis. The discrete PCR product band, visualized upon ethidium bromide staining, was excised from the gel, and the DNA fragment therein was isolated by spin filtration. After BamHI/EcoRI digestion, the PCR product was subcloned into the BamHI/EcoRI site of pGEX-2TK and transformed into E. coli BL21. To verify its authenticity, the cDNA insert was subjected to nucleotide sequencing as described above.

For the human SULT1C sulfotransferase 2, the nucleotide sequences encoding the N-terminal and C-terminal portions, as defined by an internal XbaI restriction site, were amplified using, respectively, the cDNA clone F-11 and the I.M.A.G.E. cDNA clone 302867 as the templates in conjunction with sequence-specific sense and antisense oligonucleotide primers shown in Table I. Experimental conditions for PCR amplifications, separation of PCR products, and subcloning into pGEX-2TK were the same as those described above, except that the two PCR products were subjected to, respectively, BamHI/XbaI and XbaI/EcoRI digestion before being tandemly subcloned into pGEX-2TK. The full-length SULT1C sulfotransferase 2 thus generated was verified by nucleotide sequencing.

Competent E. coli BL21 cells were transformed with pGEX-2TK harboring the full-length cDNA encoding the human SULT1C sulfotransferase 1 or 2. The transformed cells, grown to an A$_{600nm}$ of 0.5 in 1 liter of LB medium supplemented with 100 μg/ml ampicillin and induced with 0.1 mM isopropyl-β-D-thiogalactopyranoside overnight at room temperature, were collected by centrifugation and homogenized in 20 ml of ice-cold STE (10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1 mM EDTA) using an Aminco French press. The crude homogenate thus prepared was subjected to centrifugation at 10,000×g for 30 min at 4 °C. The supernatant collected was fractionated using 0.5 ml of glutathione-Sepharose, and the bound fusion protein was treated with 2 ml of a thrombin digestion buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, and 2.5 mM CaCl$_2$) containing 5 unit/ml bovine thrombin. After a 1-h incubation at room temperature with constant agitation, 5 mg of aprotinin were added to inactivate thrombin, and the preparation was subjected to centrifugation. The recombinant enzyme present in the supernatant collected was examined with respect to its enzymatic and immunologic properties.

**Enzymatic Assay—**Sulfotransferase activities of purified recombinant human SULT1C sulfotransferases were assayed using 3'-phosphoadenosine 5'-phosphosulfate as the sulfate donor. The standard assay mixture, with a final volume of 30 μl, contained 50 mM Taps-NaOH, pH 8.0, 1 mM dithiothreitol, 14 μM 3'-phosphoadenosine 5'-phosphosulfate (PAPS), and 20 μl of crude homogenate. The assay was incubated at 37 °C for 45 min, and the reaction was stopped by the addition of 15 μl of 1 M HCl. The reaction products were separated on a 12% polyacrylamide gel containing 8 M urea and visualized by autography.
Molecular Cloning of Novel Human SULT1C Sulfotransferases

**RESULTS AND DISCUSSION**

Previous studies have demonstrated that N-hydroxylation under the action of cytochrome P-450 represents the first step in the metabolic activation of arylamines or arylamines (33). N-Arylhydroxylated arylamines or arylamines that are formed can subsequently be sulfonated, generating products that are unstable and may degrade into highly carcinogenic/mutagenic electrophilic cations (6–10). It has been demonstrated that the O-sulfonation activity in experimental animals correlated to the carcinogenic potency of N-OH-2AAF (34). Investigation into the responsible enzyme in rat liver first revealed the aryl sulfotransferase IV (SULT1A1) to display activity toward N-OH-2AAF (17, 18). A SULT1C sulfotransferase was later purified, characterized, and cloned from rat liver (19, 20). The recombinant SULT1C sulfotransferase expressed in COS-1 cells was shown to exhibit a higher N-OH-2AAF-sulfonating activity than the SULT1A1 sulfotransferase (20). Attempts to identify a homologous SULT1C sulfotransferase in human liver, however, have so far yielded no clear results (24). Although the human SULT1C orthologue is still being sought after, two nucleotide sequences with high enough homology so as to be categorized in the SULT1C sulfotransferase subfamily (cf. Fig. 3) have been deposited in the EST data base (22, 23). Of these two new human sulfotransferase cDNAs (designated, respectively, 1 and 2), the SULT1C sulfotransferase 1 is a full-length clone, and the SULT1C sulfotransferase 2 is a truncated clone. In view of the potential differences between humans and rodents with regard to their constituent cytosolic sulfotransferases, we decided to clone these two novel human SULT1C sulfotransferases and express them for functional characterization.

**Molecular Cloning of a Novel Human SULT1C Sulfotransferase 2**—Analysis of the nucleotide and deduced amino acid sequences of the I.M.A.G.E. cDNA clone 302867 revealed the cDNA to encode only the C-terminal portion of a novel SULT1C sulfotransferase. As described under "Experimental Procedures," we have prepared a DNA probe and used it to screen a human fetal lung cDNA library. A positive clone encompassing the 5′ region coding for the N-terminal portion was obtained. The nucleotide sequence determined for this latter cDNA clone, spanning nucleotide residues 1–1034 of the full-length sequence shown in Fig. 1, overlaps with that of the I.M.A.G.E. cDNA clone 302867, which spans nucleotide residues 849–2143. The authenticity of the full-length sequence was confirmed by the generation of a specific PCR product in a reverse transcriptase-PCR experiment using human fetal lung total RNA as the template in conjunction with sense and antisense oligonucleotide primers corresponding to the 5′- and 3′-terminal regions of the full-length sequence. As shown in Fig. 1, the phospho-[35S] (15 Ci/mmol), and 50 μM substrate (N-OH-2AAF, p-nitrophenol, or dopamine). The reaction was started by the addition of the enzyme preparation and allowed to proceed for 15 min at 37 °C. At the end of the reaction, the reaction mixture was directly spotted on a cellulose thin-layer chromatography plate and subjected to the analysis described previously (18). It has been demonstrated that the O-sulfonation activity in experimental animals correlated to the carcinogenic potency of N-OH-2AAF (34). Investigation into the responsible enzyme in rat liver first revealed the aryl sulfotransferase IV (SULT1A1) to display activity toward N-OH-2AAF (17, 18). A SULT1C sulfotransferase was later purified, characterized, and cloned from rat liver (19, 20). The recombinant SULT1C sulfotransferase expressed in COS-1 cells was shown to exhibit a higher N-OH-2AAF-sulfonating activity than the SULT1A1 sulfotransferase (20). Attempts to identify a homologous SULT1C sulfotransferase in human liver, however, have so far yielded no clear results (24). Although the human SULT1C orthologue is still being sought after, two nucleotide sequences with high enough homology so as to be categorized in the SULT1C sulfotransferase subfamily (cf. Fig. 3) have been deposited in the EST data base (22, 23). Of these two new human sulfotransferase cDNAs (designated, respectively, 1 and 2), the SULT1C sulfotransferase 1 is a full-length clone, and the SULT1C sulfotransferase 2 is a truncated clone. In view of the potential differences between humans and rodents with regard to their constituent cytosolic sulfotransferases, we decided to clone these two novel human SULT1C sulfotransferases and express them for functional characterization.

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**Western Blot Analysis**—Purified recombinant SULT1C sulfotransferases, solubilized in SDS sample buffer and heated for 3 min at 100 °C, were subjected to SDS-polyacrylamide gel electrophoresis on a 12% polyacrylamide gel and electrotransferred onto an Immobilon-P membrane (29). The blotted membrane was blocked with 5% nonfat dried milk in phosphate-buffered saline for 1 h and probed with 20 μl of anti-rat liver SULT1C1 sulfotransferase antiserum. After a 1-h incubation, the membrane was washed with phosphate-buffered saline, treated with horseradish peroxidase-conjugated secondary antibody in phosphate-buffered saline containing 5% nonfat dried milk, and subsequently subjected to autoradiography. Autoradiography was then performed on the processed membrane.

**Northern Blot Analysis**—A human mRNA master blot (CLONTECH) was prehybridized at 65 °C for 1 h in the ExpressHyb solution (CLON-TECH) and hybridized with the human SULT1C sulfotransferase 2 cDNA probe. The probe was random-primed-labeled with [α-32P]dCTP (25) first and then hybridized to the blot. After an overnight hybridization at 65 °C, the membrane blot was washed once in 2× SSC containing 0.1% SDS for 1 h at room temperature and twice in 0.1× SSC for 45 min at 65 °C and subjected to autoradiography.

**Miscellaneous Methods**—Phosphoadenosine 5′-phosphate [35S]sulfate (15 Ci/mmol) was synthesized from ATP and [35S]sulfate using ATP sulfurylase and APS kinase from Bacillus stearothermophilus as described previously (30). SDB-polyacrylamide gel electrophoresis was performed on 12% polyacrylamide gels using the method of Laemmli (31). Protein determination was based on the method of Bradford (32), with bovine serum albumin as the standard.
open reading frame, beginning at base residue 330, encompasses 906 nucleotides and encodes a 302-amino acid polypeptide. The predicted molecular weight, 35,533, falls within the molecular weight range (33,000–36,000) generally found for cytosolic sulfotransferase enzymes (1–3). The termination codon, located at nucleotide residues 1,236–1,238, is followed by a 905-nucleotide 3'-untranslated sequence that includes a poly(A) tract. Two polyadenylation signals (AATAAA) (35), located 670 and 59 nucleotides, respectively, upstream from the poly(A) tract are found. Sequence analysis revealed the deduced amino acid sequence of this new human SULT1C sulfotransferase (designated 2) cDNA to display 62.9, 63.1, 63.1, and 62.5% identity to the amino acid sequences of the human SULT1C sulfotransferase 1 (22), rat SULT1C1 sulfotransferase (3), and rabbit SULT1C sulfotransferase.4 The high percent identity value indicates that the rabbit SULT1C sulfotransferase is likely an orthologue of the human SULT1C sulfotransferase 1.

Fig. 2 shows the amino acid sequence alignment of the human SULT1C sulfotransferases with rat and mouse SULT1C1 sulfotransferases, as well as rabbit SULT1C sulfotransferase. Based on the sequence analysis data presented above, these SULT1C sulfotransferases can now be categorized into distinct phylogenetic groups as shown in the dendrogram presented in Fig. 3.

Expression, Purification, and Characterization of the Recombinant Human SULT1C Sulfotransferases 1 and 2—The full-length cDNAs encoding the human SULT1C sulfotransferases 1 and 2 were individually subcloned into pGEX-2TK, a prokaryotic expression vector, for the expression of recombinant enzymes in E. coli. As shown in panel A of Fig. 4, recombinant human SULT1C sulfotransferases 1 and 2, cleaved from their respective fusion proteins fractionated from the E. coli homogenates using glutathione-Sepharose, were shown to migrate at, respectively, 34- and 35-kDa positions upon SDS-polyacrylamide gel electrophoresis. The recombinant enzymes thus pre-
pared were examined with respect to the immunologic cross-reactivity toward the antiserum against the rat liver SULT1C1 sulfotransferase. As shown in panel B of Fig. 4, the 34-kDa recombinant human SULT1C sulfotransferase 1 displayed a strong cross-reactivity toward the antiserum. The 35-kDa recombinant human SULT1C sulfotransferase 2, to a lesser extent, also displayed cross-reactivity toward the antiserum. The two recombinant SULT1C sulfotransferase enzymes were subjected to functional characterization with respect to their sulfotransferase activities. The activity data compiled in Table II revealed that both enzymes encoded by recombinant clones 1 and 2 catalyzed the sulfonation of p-nitrophenol but not dopamine. More importantly, the two enzymes displayed strong activities catalyzing the sulfonation of N-hydroxy-2-acetylaminofluorene. This marks the first time hydroxyarylamide or hydroxamic acid sulfotransferase activities have been shown to be associated with distinct human sulfotransferases.

**Tissue Specificity of the Expression of the New Human SULT1C Sulfotransferases—** To find clues to its functional involvement in vivo, the tissue-specific expression of the human SULT1C sulfotransferase 2 in different human tissues/organs was examined by employing the Northern blotting technique. It is pointed out that previous studies have demonstrated that human SULT1C sulfotransferase 1 is expressed in adult human stomach, kidney, and thyroid, as well as fetal kidney and liver (22). By employing the reverse transcriptase-PCR technique, we were able to amplify SULT1C sulfotransferase 1-specific sequence using total RNA from human liver, lung, or intestine as the template (data not shown). The tissue-specific expression of human SULT1C sulfotransferases 2 was examined using a CLONTECH human mRNA master blot with the cloned cDNA as probes. As shown in Fig. 5, among the poly(A)^1 RNA isolated from 50 human tissues, SULT1C sulfotransferase 2 was found to be expressed at higher levels in fetal lung and kidney and at lower levels in fetal heart, adult kidney, ovary, and spinal cord. These results on the tissue-specific expression may provide a foundation for the future investigation into the functional relevance of these new SULT1C sulfotransferases.

### Table II
Specific activities of recombinant human SULT1C sulfotransferases 1 and 2 with different substrate compounds

| Substrate Compound | Specific Activity SULT1C ST 1 | Specific Activity SULT1C ST 2 |
|--------------------|-----------------------------|-----------------------------|
| p-Nitrophenol      | 84.07 ± 1.13                | 75.34 ± 3.09                |
| Dopamine           | ND*                         | ND*                         |
| N-OH-2AAF          | 28.61 ± 2.68                | 47.50 ± 1.76                |

*Not detected.

**FIG. 4.** Western blot analysis for the immunologic cross-reactivity of the new human SULT1C sulfotransferases toward the antiserum against the rat liver SULT1C1 sulfotransferase. Panel A shows the SDS gel electrophoretic patterns of purified human SULT1C sulfotransferase 1 (lane 1) and human SULT1C sulfotransferase 2 (lane 2). Panel B shows the autoradiograph taken from the Immobilon-P membrane used in the Western blot analysis. Samples analyzed were rat liver homogenate (lane 1), purified human SULT1C sulfotransferase 1 (lane 2), and purified human SULT1C sulfotransferase 2 (lane 3).

**FIG. 5.** Dot blot analysis of human SULT1C sulfotransferase 2 mRNA. A, diagram showing the loading pattern for the CLONTECH human mRNA master blot. B, dot blot analysis of the human mRNA master blot using human SULT1C sulfotransferase 2 cDNA as a probe. Panel B shows the autoradiograph taken from the human mRNA master blot following the analysis.
transf erase s in different human tissues/organisms. Because the SULTIC sulfotransferase enzymes are hypothesized to be involved in the bioactivation of N-hydroxylated metabolites of carcinogenic/mutagenic arylamines and arylamides (17–21), it will also be interesting to examine their relative abundance in normal versus tumor tissues.

The results derived from the present study revealed that, in humans, there are multiple SULTIC sulfotransferases present in different tissues at possibly different developmental stages. These enzymes are likely to play essential, yet unknown, physiological roles at the developmental stages in the tissues where they are expressed. These enzymes, on the other hand, may also exhibit fortuitous sulfonating activities toward xenobiotic compounds such as arylamines or arylamides following their N-hydroxylation, thereby generating reactive intermediates that are capable of causing cytotoxic and genotoxic damage to cells in vitro. Such potential cytotoxic/genotoxic effects may pose a serious risk in view of the increasingly complex dietary food and the worsening environmental contamination. Another important issue is related to the species-specific differences in terms of the tissue specificity of the expression of the SULTIC sulfotransferase enzymes, which was first indicated indirectly by the differential O-sulfonating activities found in the livers of humans and experimental animals (38). One striking example is the difficulty in demonstrating the expression of the human orthologue of the rat liver SULTIC1 sulfotransferase. Futile attempts had previously been made to detect the specific mRNA in human liver, even though the sequence is now known to be present in the human genome (24). That the mouse SULTIC1 sulfotransferase is expressed in olfactory cells (39) but not (at detectable level) in liver cells,5 seems to indicate possibly the different tissue specificity of the expression of orthologous SULTIC1 sulfotransferases that function perhaps in distinct physiologic context of individual animal species. On the other hand, however, the rabbit orthologue, with a similar organ (stomach) specificity of expression, of the human SULTIC sulfotransferase 1 investigated in the present study has recently been reported.3 More studies will be needed to clarify the similarities and differences between humans and other mammalian species in terms of their constituent sulfotransferase enzymes.

Finally, it may be worthwhile mentioning that, compared with cytochromes P-450, cytosolic sulfotransferases, which were previously known as a group of Phase II drug-metabolizing enzymes, have received considerably less attention, and much fewer of them have been identified to date. The advancement of sulfotransferase molecular biology has allowed the convenient identification of potential sulfotransferase enzymes by searching through, in particular, the EST data base for the signature sequences (23, 37) found for all known sulfotransferases. As the EST project continues to progress, it is anticipated that new members of this group of enzymes, which play important roles in not only the metabolism of drugs and xenobiotics but also the biotransformation of a variety of endogenous compounds, are bound to emerge. As exemplified by our present study, the major challenge then will probably be to elucidate the enzymatic characteristics and physiological involvements of these new sulfotransferase enzymes.

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Molecular Cloning, Expression, and Characterization of Novel Human SULT1C Sulfotransferases That Catalyze the Sulfonation of N-Hydroxy-2-acetylaminofluorene
Yoichi Sakakibara, Ken Yanagisawa, Junko Katafuchi, David P. Ringer, Yasunari Takami, Tatsuo Nakayama, Masahito Suiko and Ming-Cheh Liu

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