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Examination of Human Tissue Cytosols for Expression of Sulfotransferase Isoform 1A2 (SULT1A2) Using a SULT1A2-Specific Antibody

Susan Nowell, Bridgett Green, Yong Ming Tang, Rick Wiese, and Fred F. Kadlubar

National Center for Toxicological Research, Jefferson, Arkansas (S.N., B.G., F.F.K.); Roswell Park Cancer Institute, Buffalo, New York (S.N.); Cedars-Sinai Medical Center, Medical Genetics Institute, Los Angeles, California (Y.M.T.); and Pierce Biotechnology, Rockford, Illinois (R.W.)

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ABSTRACT

Sulfotransferase isoform 1A2 (SULT1A2) is a member of the cytosolic sulfotransferase family of phase II detoxification enzymes. Studies with recombinant enzymes have shown that SULT1A2 can catalyze the bioactivation of several procarcinogens, indicating a potential role in chemical carcinogenesis. However, previous studies have suggested that the SULT1A2 transcript has a splicing defect that might prevent it from becoming translated into protein; therefore, we sought to determine the expression of SULT1A2 in tissues. An antibody directed against a region of human SULT1A2 that differs from other known sulfotransferase isoforms was developed and used to screen a large number of cytosolic fractions from various tissues. Although the SULT1A2 antibody recognized recombinant SULT1A2 and did not cross-react with other SULT isoforms, the expression of SULT1A2 was not detected in any tissue examined. These studies suggest that if SULT1A2 is expressed as protein, the levels are very low and that SULT1A2 probably does not play a physiological role in chemical carcinogenesis.

Human cytosolic sulfotransferases (SULTs) are phase II detoxification enzymes that catalyze the biotransformation of a host of endogenous and exogenous substrates (Jakoby and Ziegler, 1990). Eleven human isoforms that are the products of 10 distinct genes have been identified to date (Glatt and Meinl, 2004a). The cytosolic SULTs are members of a gene superfamily that can be divided into two major subfamilies, the phenol sulfotransferases (SULT1) and the hydroxysteroid (SULT2) sulfotransferases. A SULT4A1 isoform has been described that has high expression and tissue selectivity for brain, although no substrate has been identified for this isoform to date (Falany et al., 2000). All cytosolic SULTs catalyze the transfer of the sulfonoxy group from the obligatory cofactor, 3′-phosphoadenosine-5′-phosphosulfate, to a wide variety of endogenous compounds (steroid and thyroid hormones and bile acids) and a host of structurally diverse xenobiotics. The net result of this reaction, in most cases, is to render the substrate more water-soluble, leading to excretion from the organism. However, in some instances, sulfation of a molecule leads to bioactivation because of the fact that the sulfate group formed by *O*-sulfonation is electron-withdrawing and can become a good leaving group, generating an electrophile capable of binding to cellular macromolecules (Minchin et al., 1992; Chou et al., 1995).

The SULT1A family consists of three members, SULT1A1, SULT1A2, and SULT1A3, which are 93 to 96% identical at the amino acid level. When recombinant enzymes are examined, SULT1A1 and SULT1A2 share overlapping substrate specificity, but the affinity for substrate and rate of reaction is an order of magnitude lower in SULT1A2.

When *Salmonella typhimurium* TA1538-derived strains expressing alloenzymes of SULT1A2 were constructed and mutagenicity assays were performed, it was reported that bioactivation of promutagens was evident in the constructs, although the magnitude of mutagenicity varied by alloenzyme and by the promutagen under investigation (Meinl et al., 2002). It was also reported that SULT1A2 was the most efficient enzyme in sulfating some aromatic hydroxylamines.

These data suggest a potential role for SULT1A2 in the mutagenicity and carcinogenicity of substrates that are widespread in our environment. Although RNA for SULT1A2 has been detected in many tissues (Dooley et al., 2000), the protein expression levels of this isoform have not been elucidated. We have generated an anti-peptide antibody that discriminates SULT1A2 from SULT1A1 and SULT1A3 and

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ABBREVIATIONS: SULT, sulfotransferase; SMCC, succinimidyl 4-(*n*-maleimidomethyl)-cyclohexane-1-carboxylate; NHS, *N*-hydroxysuccinimide; MOPS, 4-morpholinepropanesulfonic acid.

have screened several human tissues for the protein expression of SULT1A2.

Materials and Methods

Materials. Recombinant SULT1A1, SULT1A2, SULT1A3, SULT2E1, and SULT2A1 were purchased from Invitrogen (Carlsbad, CA), as well as 12% NuPAGE gels, MOPS electrophoresis running buffer, nitrocellulose, and MOPS transfer buffer. DiscoverLight Arrays and Western blotting detection reagents were purchased from Pierce Biotechnology Inc. (Rockford, IL). Human tissues were obtained from the U.S. Cooperative Human Tissue Network (National Cancer Institute, Bethesda, MD).

Peptide Selection and Antiserum Production. A sequence region of SULT1A2 that is distinguishable from SULT1A1 was identified by sequence comparison. This region began at amino acid residue 147 with the peptide sequence KVYPHPGTWESFC and differed from SULT1A1 and SULT1A3 (the most structurally similar SULTs) sequence by three amino acids. The first 12 amino acid residues are specific to SULT1A2; the C-terminal cysteine residue was designed to couple the peptide to the carrier protein. The antigenicity and the surface possibility of the region was analyzed and predicted by the computer software package MacVector (International Biotechnologies, New Haven, CT). The Kyte-Doolittle method was used in the calculation over a window of seven residues. The peptide was synthesized on an Advanced Chemtech peptide synthesizer by Alpha Diagnostic Inc. (San Antonio, TX) using solid-phase chemistry. The synthesized peptide was then coupled to a carrier protein (keyhole limpet hemocyanin) through the cysteine residue at the C terminus of the peptide using *N*-succinnidyl bromoacetate as a cross-linking reagent (Bernatowicz and Matsueda, 1986). Specifically, succinimidyl 4-(*n*-maleimidomethyl)-cyclohexane-1-carboxylate (SMCC) and its water-soluble analog Sulfo-SMCC, heterobifunctional cross-linkers that each contain an *N*-hydroxysuccinimide (NHS) ester and a maleimide group, were used in the coupling reaction. NHS esters react with primary amines at pH 7 to 9 to form covalent amide bonds. Hydrolysis of the NHS ester, which is a competing reaction, increases with increasing pH and decreasing protein concentrations. Maleimides react with sulfhydryl groups at pH 6.5 to 7.5 to form stable thioether bonds. At pH values >7.5, reactivity toward primary amines and hydrolysis of the maleimide group can occur; however, the maleimide groups of Sulfo-SMCC and SMCC are unusually stable up to pH 7.5. For conjugation, the NHS ester was reacted first, excess reagent was removed, and then the sulfhydryl-containing molecule was added. SMCC was dissolved in dimethyl sulfoxide or and added to the reaction mixture at a final solvent concentration of 10% to minimize detrimental affects to the protein.

Rabbit immunization was performed at Alpha Diagnostics and has been described previously (Tang et al., 1999). For purposes of comparison, an anti-peptide antibody generated against SULT1A1 at the same sequence (beginning at amino acid 147: KVHPEPGTWDSF) was also produced (data not shown). Antibodies were used in assays without further purification.

Cytosol Preparation. Cytosolic fractions were prepared from human tissues using the same method for preparation of microsomal fractions that has been described previously (Tang et al., 1999) with minor modifications. Briefly, the supernatant from the 105,000*g* spin was aliquoted into tubes, flash-frozen in liquid nitrogen, and stored at −80°C until Western blot analysis was performed.

Western Blot Analysis. Proteins (100 μg of each sample) were electrophoresed and transferred to nitrocellulose, blocked, and incubated overnight with the anti-SULT1A2 antibody. The blots were developed using reagents from Pierce Biotechnology according to the manufacturer's directions using a horseradish peroxidase-conjugated secondary antibody. Chemiluminescent detection was per-

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SULT1A1  MELIQDTSRP PLEYVKGVPL IDYFAEALGP LQSFGARPDD LLISTYPKSG  (50)
SULT1A2  MELIQDISRP PLEYVKGVPL IKYFAEALGP LQSFQARPDD LLISTYPKSG
SULT1A3  MELIQDTSRP PLEYVKGVPL IKYFAEALGP LQSFQARPDD LLINTYPKSG

SULT1A1  TTWVSQILDM IYGGGDLEKC HRAPIFMRVP FLEFKAPGIP SGMETLKDTP  (100)
SULT1A2  TTWVSQILDM IYGGGDLEKC HRAPIFMRVP FLEFKVPGIP SGMETLKNTP
SULT1A3  TTWVSQILDM IYGGGDLEKC NRAPIYVRVP FLEVNDPGEP SGLETLKDTP

SULT1A1  APRLLKTHLP LALLPQTLLD QKVKVVYVAR NAKDVAVSYY HFYHMAKVHP  (150)
SULT1A2  APRLLKTHLP LALLPQTLLD QKVKVVYVAR NAKDVAVSYY HFYHMAKVYP
SULT1A3  PPRLIKSHLP LALLPQTLLD QKVKVVYVAR NPKDVAVSYY HFHRMEKAHP

SULT1A1  EPGTWDSFLE KFMVGEVSYG SWYQHVQEWW ELSRTHPVLY LFYEDMKENP  (200)
SULT1A2  HPGTWESFLE KFMAGEVSYG SWYQHVQEWW ELSRTHPVLY LFYEDMKENP
SULT1A3  EPGTWDSFLE KFMAGEVSYG SWYQHVQEWW ELSRTHPVLY LFYEDMKENP

SULT1A1  KREIQKILEF VGRSLPEETV DFMVQHTSFK EMKKNPMTNY TTVPQEFMDH  (250)
SULT1A2  KREIQKILEF VGRSLPEETV DLMVEHTSFK EMKKNPMTNY TTVRREFMDH
SULT1A3  KREIQKILEF VGRSLPEETM DFMVQHTSFK EMKKNPMTNY TTVPQELMDH

SULT1A1  SISPFMRKGM AGDWKTTFTV AQNERFDADY AEKMAGCSLS FRSEL       (295)
SULT1A2  SISPFMRKGM AGDWKTTFTV AQNERFDADY AEKMAGCSLS FRSEL
SULT1A3  SISPFMRKGM AGDWKTTFTV AQNERFDADY AEKMAGCSLS FRSEL
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Fig. 1. Amino acid alignment of SULT1A1, SULT1A2, and SULT1A3. The amino acid sequences of SULT1A2 and SULT1A3 compared with SULT1A1. Red letters denote differences in sequence between SULT1A1 and SULT1A2. Blue letters denote differences in sequence between SULT1A1 and SULT1A3. The highlighted and underlined section indicates the peptide sequence of SULT1A2 used in generating the anti-peptide antibody.

formed with Chemi-glow (Alpha Innotech, San Leandro, CA) detection reagents. The blots were visualized with an Alpha Innotech model 8900 Imager. Duplicate blots were performed with the SULT1A1 anti-peptide antibody.

Tissue Lysate Arrays. DiscoverLight array kits were used to screen for proteins showing immunoreactivity with antibodies to either SULT1A1 or SULT1A2, according to the manufacturer's directions. In cases in which there was potential immunoreactivity with anti-SULT1A2, individual tissue lysates were purchased from Pierce Biotechnology and Western blotted to confirm immunoreactivity.

Results

SULT1A2-Specific Peptide Selection and Characterization of Antisera. Sequence alignment data from Fig. 1 was used to select an antigenic epitope with a sequence significantly different from either SULT1A1 or SULT1A3, the two isoforms with structural similarity to SULT1A2. The peptide was predicted to have a high degree of hydrophilicity, surface probability, and antigenicity according to computer modeling (Fig. 2). The purity of the peptide, as determined by high-performance liquid chromatography, was greater than 85% (data not shown). The peptide was coupled to the carrier protein (keyhole limpet hemocyanin) via the cysteine residue at the C terminus of the peptide and was used to immunize New Zealand White rabbits. Antisera produced against this peptide had a titer of approximately 10^5, as determined by enzyme-linked immunosorbent assay.

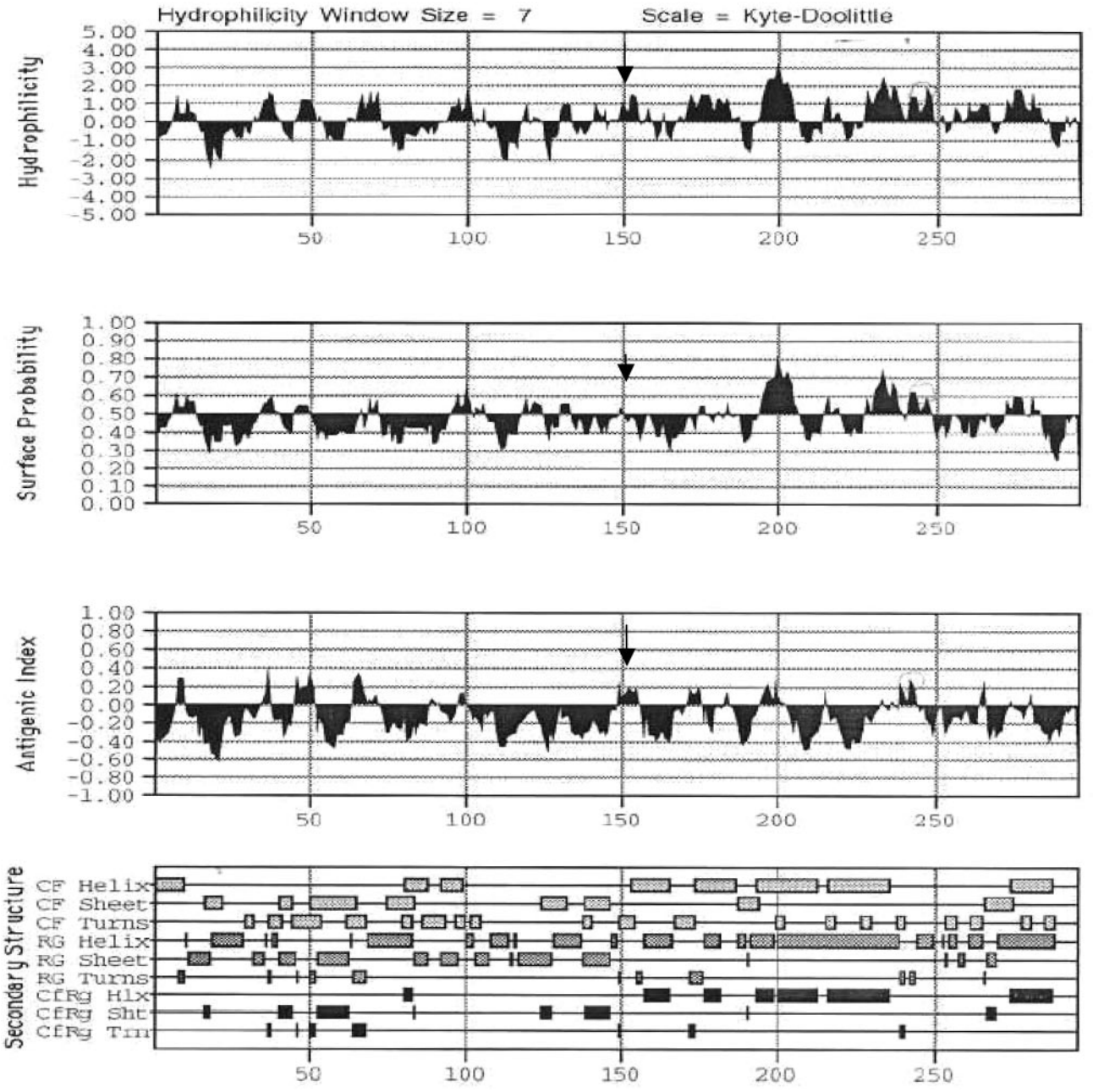

Fig. 2. Hydrophilicity, surface probability, and antigenicity of human SULT1A2. The amino acid sequence of SULT1A2 was analyzed by the program MacVector, and the hydrophilicity, antigenicity, and surface probability of the whole enzyme were predicted. Arrows indicate the selected region for peptide production.

Specificity and Sensitivity of the SULT1A2 Antibody. Potential cross-reactivity of the antibody with other known SULT isoforms was investigated using recombinant enzymes. Immunoblotting was performed using recombinant SULT1A1, SULT1A2, SULT1A3, SULT1E1, SULT2A1, and control protein supplied by the manufacturer. The dilution of the primary antibody was 1:200, and the blots were incubated overnight at 4°C; the secondary antibody dilution was 1:10,000. Figure 3A shows that when 50 μg of recombinant enzyme is used, there is no apparent cross-reactivity with these other SULT isoforms.

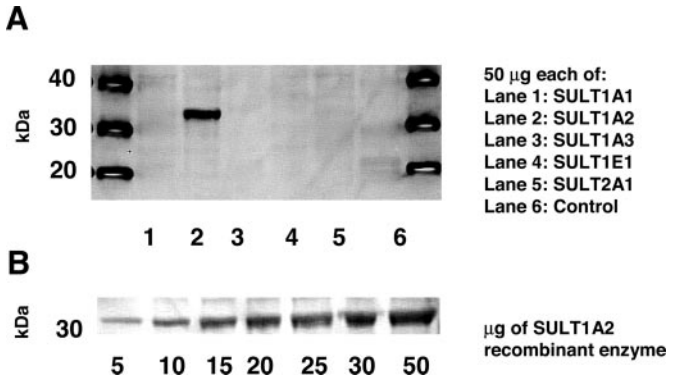

Fig. 3. Western blot analysis of recombinant sulfotransferases with anti-SULT1A2. A, cross-reactivity of the antibody with other SULT isoforms was determined using 50 μg of recombinant enzyme and an overnight incubation with the primary antibody. B, concentration-dependent detection of recombinant SULT1A2 was determined using varying amounts of enzyme and a 2-h incubation with the primary antibody.

TABLE 1
Human tissue cytosols screened for SULT1A2 protein expression

Tissue Cytosol	Number	Immunoreactivity	
		SULT1A1	SULT1A2
Bone	2	+	N.D.
Colon	35	+	N.D.
Duodenum	10	N.D.	N.D.
Esophagus	6	N.D.	N.D.
Liver	52	+	N.D.
Lung	16	N.D.	N.D.
Pancreas	22	+	N.D.
Prostate	34	+	N.D.
Stomach	7	N.D.	N.D.
Small Intestine	32	+	N.D.

N.D., not detected; +, present.

A 5- to 50-μg sample of recombinant SULT1A2 was transferred to nitrocellulose and exposed to the SULT1A2 antibody for 2 h at room temperature. As shown in Fig. 3B, immunoreactive bands were detected at each concentration of recombinant enzyme tested. Other experiments using lesser amounts of enzyme (0.1–5 μg) indicated that SULT1A2 recombinant protein could be detected at levels as low as 0.5 μg. When recombinant SULT1A1 was probed with the anti-SULT1A1 antibody, the limit of detection of this antibody was identical with that found for the SULT1A2 antibody/recombinant enzyme (data not shown).

Screening of Tissues for SULT1A2 Expression. A 100-μg sample of each cytosol prepared from several human tissues was immunoblotted and probed for the presence of SULT1A2. Table 1 lists the different tissues and the numbers of individual samples from each type of tissue examined. Examination of 52 individual liver samples failed to show immunoreactivity for SULT1A2. Likewise, there was no immunoreactivity in 35 colon samples, 34 prostate samples, and 32 small intestine samples. Although fewer numbers of individual samples from other tissues were available, examination of these tissues also failed to detect SULT1A2 expression.

SULT1A2 Expression Screening Using the DiscoverLight Array Kit. To expand the number of tissues examined and to include tumor tissue, the DiscoverLight tissue array was probed for immunoreactivity with the SULT1A2 antibody (Fig. 4B). The array consists of pooled samples from 17 different organs and includes both tumor and normal tissue from each organ. These samples are arrayed on a nitrocellu-

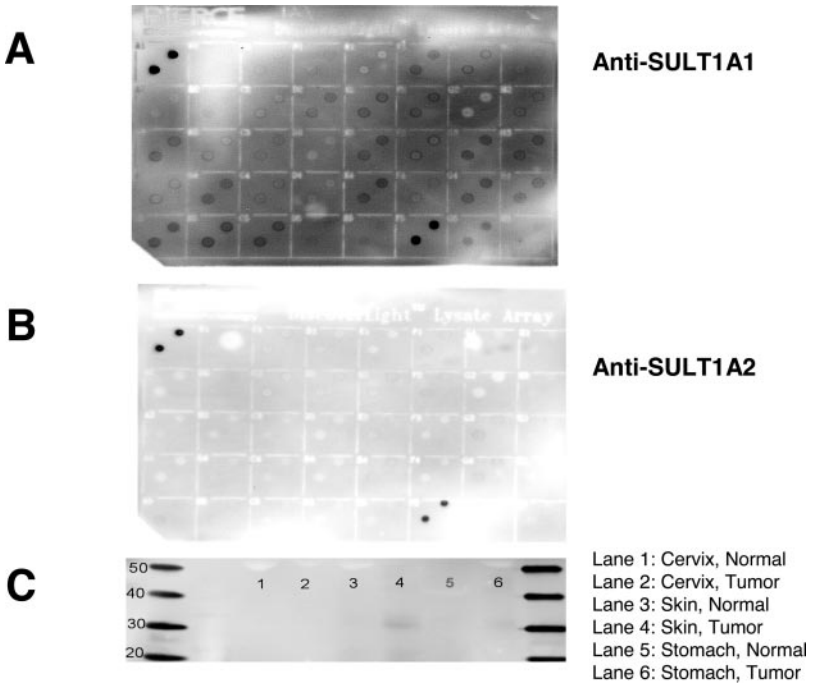

Fig. 4. Tissue lysate array probed with (A) anti-SULT1A1 antibody or (B) anti-SULT1A2 antibody. Lysate arrays were incubated overnight with the antibodies and developed with SuperSignal West Femto detection reagents for SULT1A2 and SuperSignal West Dura detection reagents for SULT1A1. C, Western blot analysis of potential positives incubated overnight with anti-SULT1A2 antibody and developed with SuperSignal West Femto detection reagents.

lose membrane that contains positive control elements in addition to the samples. As a control, a duplicate array was probed for immunoreactivity with an antibody specific for SULT1A1 (Fig. 4A). Table 2 lists the array positions, tissue types, and a summary of immunoreactivity with either SULT1A1 or SULT1A2 antibodies. SULT1A1 expression was found, to varying degrees, in most of the tissues on the array. SULT1A2 expression, on the other hand, was not high in any tissue, but potential positives were detected with samples from the cervix, skin, and stomach. To confirm these potential positives, Western blot analysis was performed using 30 μg of the tissue lysates and the most sensitive detection reagent available (SuperSignal West Femto; Pierce Biotechnology), which can detect low femtogram amounts of proteins. A very faint immunoreactive band was evident in the skin tumor lysate, but the other lysates tested showed no immunoreactivity (Fig. 4C).

Discussion

SULT1A2 is a member of the phenol sulfotransferase subfamily of cytosolic sulfotransferases. Transcript for this isoform has been identified, but to date, detection of SULT1A2 as a protein in human tissues has not been reported. Using an anti-peptide antibody specific for SULT1A2, we examined more than 200 cytosolic fractions from 10 different tissues and found no evidence of immunoreactive protein. When a commercially available tissue lysate array containing 17 normal and 17 tumor pooled tissue lysate elements was screened for SULT1A2, potential immunopositive tissues were identified. Western blotting was performed to confirm this finding, but immunostaining in the potentially positive tissues was too low to be conclusive.

TABLE 2
DiscoverLight Array screened for SULT1A2 immunoreactivity

Array Position	Tissue Sample Type	SULT1A1 Antibody	SULT1A2 Antibody
A1	Control	++++	++++
A2	Adrenal, normal	+	–
A3	Adrenal, tumor	++	–
A4	Bladder, normal	+	–
A5	Bladder, tumor	+++	–
B1	Brain, normal	–	–
B2	Brain, tumor	–	–
B3	Breast, normal	–	–
B4	Breast, tumor	++	–
B5	Cervix, normal	+++	?
C1	Colon, normal	+	–
C2	Colon, tumor	++	–
C3	Esophagus, normal	+	–
C4	Esophagus, tumor	+	–
C5	Cervix, tumor	+++	?
D1	Kidney, normal	–	–
D2	Kidney, tumor	+	–
D3	Liver, normal	+	–
D4	Liver, tumor	+	–
D5	A431 cell line	+	–
E1	Lung, normal	–	–
E2	Lung, tumor	++	–
E3	Ovary, normal	++	–
E4	Ovary, tumor	+++	–
E5	HeLa cell line	–	–
F1	Prostate, normal	++	–
F2	Prostate, tumor	++	–
F3	Rectum, normal	–	–
F4	Rectum, tumor	–	–
F5	Control	++++	++++
G1	Skin, normal	+	?
G2	Skin, tumor	–	?
G3	Stomach, normal	++	?
G4	Stomach, tumor	++	?
G5	MCF-7 cell line	++	–
H1	Thyroid, normal	–	–
H2	Thyroid, tumor	+	–
H3	Uterus, normal	++	–
H4	Uterus, tumor	++	–
H5			

+, present; –, absent; ?, ambiguous.

Previous studies have shown that SULT1A2 shares similar substrate specificity with SULT1A1, although K_m values are generally an order of magnitude higher with SULT1A2 compared with SULT1A1 (Raftogianis et al., 1999). Raftogianis et al. determined that in the liver, SULT1A2 did not contribute to the "thermostable phenol sulfotransferase" phenotype because of the lack of detectable activity when assay conditions were optimal for SULT1A2. When SULT1A2 alloenzymes were expressed in *S. typhimurium* and metabolic activation of several aromatic amines was examined, in some cases, SULT1A2-expressing cells were more efficient at activation than SULT1A1 (Meinl et al., 2002; Glatt and Meinl, 2004b). Additionally, when SULT1A2 is expressed in Chinese hamster V79 cells, it is capable of bioactivating 3-nitrobenzanthrone and its metabolites (Arlt et al., 2003). These findings suggest a potential role of SULT1A2, if it is expressed as a functional protein, in the chemical carcinogenesis of these agents. However, it has been suggested that a splicing defect in SULT1A2 prevents its expression as a functional protein, in which case SULT1A2 would effectively be considered a pseudogene (Dooley et al., 2000).

SULT1A2 genetic polymorphisms have been identified (Raftogianis et al., 1999), and recently, these polymorphisms have been investigated in relation to cancer risk. Studies by Peng et al. (2003) showed that, as previously reported, *SULT1A1*1* and *SULT1A2*1* alleles were in positive linkage disequilibrium but showed no association with risk of liver, colon, lung, oral, gastric, renal, or cervical cancer in a Taiwanese population. Another study examining breast cancer risk in Chinese women indicated that *SULT1A2* polymorphisms were not associated with breast cancer risk but might be associated with early onset of disease (Hou et al., 2002). However, because polymorphisms in *SULT1A1* and *SULT1A2* are linked, it is possible that the observed association is actually caused by *SULT1A1* alleles.

Given these emerging molecular epidemiological studies, it is important to determine whether SULT1A2 is expressed as a protein in human tissues. We therefore designed an anti-peptide antibody specific for human SULT1A2 and surveyed several normal and tumor tissues. In cytosols prepared from human tissues, we did not detect SULT1A2 immunoreactivity. However, when a tissue lysate array was probed with anti-SULT1A2 and developed with a chemiluminescent substrate designed to detect immunoreactive proteins present in the femtogram range, we identified potential positives in cervix, skin, and stomach. To confirm these findings, we then performed Western blot analysis of both tumor and normal lysates from these tissues. We found that there was a weak immunopositive reaction in skin tumor but not in the other lysates. However, the low degree of reaction was not convincing of expression. In any case, the antibody failed to detect expression of SULT1A2 in all other cytosols and tissue lysates examined. Anti-peptide antibodies in general are not as avid as antibodies directed against whole proteins, so it is

possible that some very low levels of SULT1A2 could be expressed under certain circumstances. However, the anti-peptide antibody directed against SULT1A1 revealed abundant expression of this isoform. Little is known about the regulation of SULT1A2; therefore, it is possible that expression is induced in some physiological states. It does not seem to be hormonally regulated because we detected no gender-related differences in expression.

In summary, we did not detect SULT1A2 expression in any tissue using a specific anti-peptide SULT1A2 antibody. These findings suggest that although SULT1A2 expression might occur very occasionally, given the fact that SULT1A1 is expressed at much higher levels and is much more metabolically active than SULT1A2, this enzyme is not likely to be physiologically relevant under normal conditions. Therefore, caution should be used in interpreting *SULT1A2* genotype/disease association studies.

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Address correspondence to: Dr. Susan Nowell, Roswell Park Cancer Institute, BSB S704, Elm and Carlton Streets, Buffalo, NY 14263. E-mail: susan.nowell@roswellpark.org