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Expression Profiling of Human Sulfotransferase and Sulfatase Gene Superfamilies in Epithelial Tissues and Cultured Cells

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The bioavailability of drugs administered topically or orally depends on their metabolism by epithelial enzymes such as the cytosolic sulfotransferases (*SULT*). Reverse transcriptase-polymerase chain reaction (RT-PCR) methods were established to detect expression of 8 *SULT* genes and 4 arylsulfatase (*ARS*) genes in human tissues of epithelial origin and in cultures of normal and transformed (cancer) cells. The results indicate: (i) *SULT 1A1, 1A3, ARSC,* and *ARSD* genes are ubiquitously expressed; (ii) expression is frequently similar between cell lines and corresponding tissues; (iii) *SULT* gene expression in normal cultured cells is generally comparable to the expression in associated transformed (cancer) cell lines; (iv) *SULT 1A1* promoter usage is mainly tissue specific; however, both promoters are frequently used in *SULT 1A3* expression; and (v) the expression profile of *SULT 1A1, 1A3, 1E1,* and *2B1a/b* suggests that one or more of these isoforms may be involved in the cutaneous sulfoconjugation of minoxidil and cholesterol. © 2000 Academic Press

Key Words: **skin; keratinocytes; melanocytes; fibroblasts; minoxidil; cholesterol sulfate; catecholamines; gastrointestinal; drug metabolism.**

The bioavailability and activity of therapeutic drugs following topical or oral administration often depends on intracellular metabolism within the epithelia. Several key drug-metabolizing gene superfamilies are present in epithelia and include isozymes of cytochrome P_{450}, glucuronosyl transferase, glutathione sulfurtransferase, and cytosolic sulfotransferase. The cytosolic sulfotransferases (*SULTs*) have been of particular interest to our laboratory in view of their critical roles in the metabolism of both xenobiotic and endogenous compounds within skin and other epithelia (1, 2). *SULTs* catalyze the transfer of the sulfonate group from phosphoadenosine phosphosulfate (PAPS) to a suitable acceptor group (typically a hydroxyl residue) on a substrate to form either a sulfate ester or a sulfamate (3). Sulfonation usually results in the bioinactivation of the substrate, but occasionally an active metabolite is produced.

The substrates recognized by the *SULTs* are structurally diverse, including various drugs, neurotransmitters, thyroid hormones, steroids, and procarcinogens, thus implicating these enzymes in numerous fundamental metabolic and physiologic processes. The enzymes have been categorized into two major classes based on amino acid sequence relatedness, substrate specificity, and genomic organization (i.e., exon structure). The two major classes are capable of sulfating either (a) phenols, catecholamines, thyroid hormones, and estrogens, or (b) hydroxysteroids such as dehydroepiandrosterone, DHEA (1–9). A superfamily of at least nine human cytosolic *SULT* genes has been discovered and cloned to date, and are summarized in Table I. The enzymes have different but overlapping substrate recognition properties, and the preferred (i.e., low K_m) substrates are noted.

Phenol sulfotransferase (PST) enzymes are expressed in many human tissues including liver, intestine, brain, adrenal gland, skin, olfactory epithelium, and platelets (10–18). PSTs play essential roles in the metabolism of various xenobiotics (i.e., planar phenols, such as naphthol and p-nitrophenol, and drugs such as acetaminophen and minoxidil) and endogenous compounds (i.e., catecholamines and thyroid hormones). The major forms of human PST are the phenol-preferring/thermostable PST, P-PST, and the monoamine neurotransmitter-preferring/thermolabile PST, M-PST (10–12, 18, 19). The M-form of this enzyme, which is abundant in the intestines, has a lower K_m of ca. 1 μM for dopamine than the P-form. The dramatic post-prandial increase in expression of M-PST in the intestine has been recently suggested to reflect a "gut-blood" barrier for detoxifying dietary biogenic amines

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TABLE I

Human Cytosolic Sulfotransferase (A) and Aryl Sulfatase (B), Gene Superfamilies, Their Gene Loci, and Preferred Substrates

A

SULT	Gene	Enzyme	Locus	Substrate	In this work
1A1	(STP1)	P-PST-1	16p	Phenols	+
1A2	(STP2)	P-PST-2	16p	Phenols (high Km)	+
1A3	(STM)	M-PST	16p	Catecholamines	+
1B1	—	ST1B2	—	Thyroid hormones	+
1C1	—	SULT1C1	2q	Hydroxyarylamines	+
1C2	—	SULT1C2	—	Hydroxyarylamines	
1E1	(STE)	EST	4q	Estrogens	+
2A1	(STD)	DHEA-ST	19q	Hydroxysteroids	+
2B1a	—	SULT2B1a	19q	Hydroxysteroids	+
2B1b	—	SULT2B1b	19q	Hydroxysteroids	+

B

Gene	Locus	Substrates	In this work
ARSA	22q	Sulfated glycolipids	
ARSB	5q	Dermatan sulfate Chondroitin sulfate	
ARSC	Xq	Cholesterol sulfate Steroid sulfates	+
ARSD	Xq	?	+
ARSDβ	Xq	?	+
ARSE	Xq	?	+
ARSF	Xq	?	

(20). P-PST, which is abundant in the liver, exhibits a K_m in the mM range (i.e., lower activity) for dopamine. The reverse is observed for the substrate p-nitrophenol.

At least one isoform of PST has been implicated in the enzymatic bioactivation of the hairgrowth-stimulating agent minoxidil (21), the active ingredient in topical Rogaine. The identity of the minoxidil-sulfating form of PST in human skin has not been rigorously established, although the most likely candidate proposed by us and other investigators is P-PST (i.e., *SULT 1A1* gene). Our prior immunohistochemical results indicated that an enzyme in rat skin homologous to human P-PST is restricted in localization to the lower outer root sheath (ORS) of actively growing anagen hair follicles in pelage skin and vibrissa (22). Apparently, a PST isozyme plays a key role in the sulfation of minoxidil within the epithelial ORS cells of the temporary portion of anagen hair follicles.

Given that minoxidil sulfate is the active metabolite in skin, it follows that the genetic variation in sulfation capacity found in the human population, as has been reported for the *SULT 1A1* gene (23), may account for the differential efficacy of topical minoxidil therapy. Although it is not yet clear which of the PST mRNAs are expressed in human hair follicles or interfollicular epidermis, it appears that multiple isozymes, including both P- and M-PST, are capable of sulfating minoxidil *in vitro* (24). While immunolocalization and *in situ* hybridization studies of human PSTs have been performed on brain, kidney, lung, liver, breast, and colon (18, 25–27), similar approaches have not been performed on human skin at present.

Numerous cDNAs encoding PSTs have been reported by various groups including our laboratory (1, 2). The *SULT 1A1*, *1A2*, and *1A3* genes, which we have mapped to the short arm of chromosome 16 (28–30), have been well characterized biochemically as recombinant proteins. Unlike the other two PST enzymes, the recombinant proteins of *SULT 1A2* cDNA alleles are significantly less active against simple phenolic substrates, and are inactive against dopamine (31, 32). No data have been published indicating the presence of *SULT 1A2* gene expression at either the mRNA or protein level in human tissues or cell lines, and it is uncertain whether the *SULT 1A2* genes actually produces a functional enzyme *in vivo*.

Among the PST family, heterogeneity has been demonstrated in the 5′ untranslated regions (UTR) of the cDNAs (30, 32–37). It is possible that the PST genes have at least two separate cis-acting promoter sequences. In the case of *SULT1A1,* one promoter appears to be proximal to exon II (containing the ATG start codon), producing an unspliced mRNA; and the other is located at an upstream site, resulting in an intron in the UTR. The coding regions of the allelic

forms of a given PST are either identical or very similar. The use of alternate promoters has been suggested to reflect tissue-specific expression, however the full consequence of their presence is uncertain.

Sulfation of the 3-OH or 17-OH moieties of steroid hormones (e.g., estrogens and DHEA) can be mediated by several of the *SULT* isozymes in both of the major gene families. *SULT 2A1* and *1E1* are considered to be two of the principal genes involved in this process in view of their efficient substrate recognition properties. The expression of these genes has been demonstrated in a small number of human tissues such as adrenal gland, liver, kidney, and mammary epithelium (38–40). Both gene products catalyze the formation of receptor-insensitive sulfate esters of steroid hormones. Using anti-DHEA-ST antisera in immunohistochemistry studies, we have determined that *SULT 2A1* is expressed in the human adrenal cortex, where it plays a role in neuroendocrine metabolism of hydroxysteroids (39). Steroid sulfates are likely to be transported outside of *SULT*-expressing cells by membrane-associated pumps, such as MDR1 P-glycoprotein or MRP (41). Subsequently, the sulfate ester must be de-sulfated by steroid arylsulfatase, *STS*, referred to as arylsulfatase C (*ARSC*) located in the target tissues to regenerate the free active steroid molecules.

Cholesterol sulfate (CS) was once thought to solely function as a lipid ingredient of the skin that aids in the prevention of transepidermal water loss. However, it also appears to play a critical role as a mediator of differentiation (42, 43). CS has been identified as an activator of several isoforms of protein kinase C (PKC) in mammalian keratinocytes (44–48). One of the CS-activated calcium-independent isozymes, PKC_{eta}, is expressed at high levels in the epidermis and lung (47, 48), but at negligible levels in many other tissues. Activation of PKC_{eta} results in the transcriptional activation of epidermal transglutaminase 1 (TGase 1) (49). TGase 1 crosslinks the protein precursors of the intracellular cornified envelope during terminal differentiation. Thus, the CS pathway, among others, plays a key role in determining the thickness of the epidermis, and in the formation of the dead stratum corneum layers.

The critical role played by CS in skin differentiation is further underscored by the discovery of the genetic basis of human X-linked ichthyosis (42). This genetic disorder produces thickening of the epidermis and associated scaling of the stratum corneum. It is caused by a mutation affecting the steroid sulfatase *ARSC*, which encodes a microsomal enzyme that removes the sulfate ester from CS (50–52). Mutations of the *ARSC* gene in X-linked ichthyosis alter the ratio of CS to free cholesterol and affect the normal differentiation profile of keratinocytes. Aberrantly high levels of CS result in the premature induction of differentiation of keratinocytes within the suprabasal layers. Since CS activates PKC_{eta}, which in turn increases TGase 1 activity, it is also interesting to note that a genetic defect in TGase 1 results in autosomal recessive lamellar ichthyosis, another epidermal differentiation disorder (49). Taken together, this biochemical and genetic evidence indicates that the cholesterol sulfate pathway (i.e., "cholesterol sulfotransferase," *ARSC*, PKC_{eta}, and TGase 1) serves a critical role in epidermal differentiation and thickness. Thus, it is of great interest to identify the *SULT* isozyme responsible for sulfonation of cholesterol.

Unlike the genes mentioned above, far less is known about *SULT 1B1, 1C1, 1C2,* and *2B1,* which have more recently been cloned. The recombinant enzymes produced by *SULT 1B1* effectively sulfates thyroid hormones (53). The *SULT 1C1* and *1C2* gene products sulfate carcinogenic hydroxyarylamines (54). The recombinant product of *SULT 2B1* recognizes hydroxysteroids similar to DHEA sulfotransferase (55). The genomic organization of *SULT 2B1* is highly similar to *SULT 2A1* and unlike that of other distantly related cytosolic *SULTs* (55). One gene encodes two *SULT 2B1* isoforms, *SULT 2B1a* and *b* that differ at the 5′-termini.

The ultimate fate of sulfonated compounds is often influenced by the action of sulfatases. Members of the sulfatase gene family catalyze sulfate hydrolysis, frequently leading to restoration of activity. As in the case of the steroid hormones, this may allow the biologically inactive form of the compound to be transported to a specific site where activity can be regained. The sulfatase gene family contains at least seven members as described in Table I. *ARSC, ARSD, ARSDβ, ARSE* and *ARSF* are capable of hydrolyzing sulfonates produced by the cytosolic *SULTs.*

Here we describe the establishment of RT-PCR methods to detect mRNA from 8 members of the *SULT* gene superfamily, *SULT 1A1, 1A2, 1A3, 1B1, 1C1, 1E1, 2A1,* and *2B1a* and *-1b,* with β-actin as a positive control. We surveyed the patterns of expression at the mRNA level of a variety of normal human epithelia and cell culture models, focusing our attention on the *SULT* genes implicated in metabolism in skin and other epithelia. Comparisons of gene expression profiles were made between normal and transformed cells *in vitro.* RT-PCR was also used to gather information regarding the use of alternate promoters for *SULT 1A1* and *1A3* as well as to detect the presence of four members of the arylsulfatase gene family that are capable of hydrolyzing products formed by the cytosolic sulfotransferases. These findings should contribute to our understanding of drug metabolism and bioavailability in human epithelia and cultured cells.

MATERIALS AND METHODS

Human tissue samples were routinely obtained as surgical-discard material. A few samples were obtained at the time of autopsy from

TABLE II

RT-PCR Primer Pairs Specific to (A) SULT and β-Actin, (B) SULT 1A1 and 1A3 Promoter Regions, and (C) ARS Genes

Gene	Amplimer (bp)	Forward primer [5′-3′]	Reverse primer [5′-3′]
		A	
1A1	122	GCAACGCAAAGGATGTGGCA (RP1)	TCCGTAGGACACTTCTCCGA (RP2a)
1A2	307	GAGGAGACTGTGGACCTCATGGTTG (RP3)	TTATTCTGGAGCCTCTTGGTCAGGC (RP4)
1A3	199	TGAGGTCAATGATCCAGGGGAA (RM1)	CGCCTTTTCCATACGGTGGAAA (RM2)
1B1	170	CAGTTCCATAGCAGACCAGATG	AATCCAGGGAGAGTCATTTCCAAC
1C1	460	GGTTTGGGGTTCCTGGTTTGAC (STC F)	GGCTGGGACTGAAGGATTGAAG (STC R)
1E1	127	TTGCCACCTGAACTTCTTCCTGCC (RE1)	TTGGATGACCAGCCACCATTAGAA (RE2)
2A1	159	TGGTTTGAAGGCATAGCTTTCC (RD1)	GGAGTGCATCAGGCAGAGAATC (RD2)
2B1a	283	ATGGCGTCTCCCCCACCTTT	CACCCACAATGGTCTCACAC
2B1b	307	CAGATCCCGGGCTTGTGGGA	CACCCACAATGGTCTCACAC
β-Actin	652	CACCCACACTGTGCCCATC (Actin1)	CTAGAAGCATTTGCGGTGG (Actin2)
		B	
1A1 distal	340	AGGCCAGGTTCCCAAGAGCT	AGTCGTGGGGCCGGTGTGTC
1A1 prox.	353	GTAAGGGAACGGGCCTGGCT	AGTCGTGGGGCCGGTGTGTC
1A3 distal	340	GACCTGATCTGGCTGTGCCA	CTGAGGGTTCCCCTGGATCA
1A3 prox.	332	ATACTCAGCCCCTGCAGGCA	CTGAGGGTTCCCCTGGATCA
		C	
ARSC	164	GGTTCCAGGCATCCTTCGTT	CTTCAAGCAGGGGCATCAGA
ARSD	201	ATGGAGGACATTTAGAGGCAAGAG	GCTGGACCACAGTAGGGAACA
ARSDβ	381	GGGCTGCCTGTTTTTCATCTC	GCTGGACCACAGTAGGGAACA
ARSDβ	381	GGGCTGCCTGTTTTTCATCTC	GAAGGAAGACAGCGTCTCTGC
ARSE	340	GTGAGAGGTTTCTGCACGCAG	AGCCACGGTCTCCAGATGTT

cadavers. The tissues were obtained from both male and female adults and were frozen at −80°C until processed for RNA isolation.

Normal human cell cultures were obtained commercially and grown as adherent monolayer cultures on disposable plastic cultureware. Normal human epidermal keratinocytes (NHEK, adult and neonatal) and normal human epidermal melanocytes (NHEM) were obtained from Clonetics for growth as submerged cultures according to manufacturer's recommendations. Normal infant human foreskin fibroblasts (HFF) were maintained in Dulbecco's modified Eagle medium (DMEM), supplemented with 10% fetal calf serum and penicillin-streptomycin. All cultures were routinely maintained in the media recommended by the supplier at 37°C in 5% CO_2.

Transformed (tumor-derived) human cell lines were obtained from American type culture collection or other sources (listed below) and grown as adherent cultures on disposable plastic cultureware. Cells were cultured in DMEM supplemented with 10% fetal calf serum and penicillin-streptomycin at 37°C in 5% CO_2, unless stated otherwise by the supplier. The following transformed human cell lines were analyzed: MS7 (primary cutaneous melanoma; c/o Paula Grammatico), SK Mel28 (metastatic cutaneous melanoma), HT1080 (fibrosarcoma), and squamous cell carcinomas (SCC) 9, 12, 15 (oral), and 13 (cutaneous) (c/o James Rheinwald).

Molecular biology methods. All methods are noted in prior publications from our laboratory or in Sambrook *et al.* (56), unless otherwise stated. Total RNA was prepared from the tissue samples and some of the cultured cells using RNeasy Total RNA isolation system according to manufacturer's instructions (Qiagen Corp.). The mRNA samples were isolated from cell cultures using the Oligotex Direct mRNA Kit (Qiagen Corp.).

Primer pairs specific to each of the 8 cytosolic *SULT* genes (*SULT 1A1, 1A2, 1A3, 1B1, 1C1, 1E1, 2A1,* and the *2B1* isoforms, *-1a* and *1b*), the proximal and distal promoter region of *SULT 1A1* and *1A3,* the four *ARS* genes (*ARSC, ARSD, ARSDβ,* and *ARSE*) and β-actin (internal positive control) were prepared to perform the reverse transcriptase-polymerase chain reactions (RT-PCR). The oligonucleotide primers were designed based on the following criteria: (i) select from regions of maximal sequence differences among the genes; (ii) the 3′ terminal nucleotide correctly matches the gene of interest, but mismatches the other genes; and (iii) primer pairs flank at least one intron, if possible. This allows the detection of unspliced pre-mRNAs or genomic DNA amplimers by electrophoresis as larger bands compared to the correct RT-PCR amplimers. The oligonucleotide primers are displayed in Table II. The expected mRNA fragment sizes are included.

RT-PCR amplifications were performed using the GeneAmp RT-PCR Kit (Perkin Elmer) and isoform specific primer pairs. The amplification reactions included an initial denaturation for 1 min, 45 s at 95°C, 35 cycles of 15 s at 95°C, and 30 s at 55°C (or 62°C for the *SULT* promoter reactions), a final extension for 7′ at 60°C and a 4°C soak. The reaction products were resolved by 3% or 4% Nusieve 3:1 agarose gel electrophoresis, stained with ethidium bromide, and photographed under UVC illumination. Relative band intensity was determined by visual inspection. β-actin was established as the reference intensity. The RT-PCR products were verified by automated DNA sequence analysis using an ABI model 377 automated DNA sequencer and Perkin Elmer sequencing reagents.

RESULTS AND DISCUSSION

The *SULT 1A1* and *1A3* genes (which produce P-PST1 and M-PST, respectively) appear to be expressed ubiquitously in human tissues based on our RT-PCR method (Table III). The available published information concerning expression at the protein level (i.e., either enzymatic or immunodetection studies) suggests their presence is manifest in a more restricted

TABLE III

RT-PCR Profiles of SULT and ARS Gene Expression in Normal Human Tissues

		Sulfotransferase									Sulfatase				
Normal tissues	Sex	1A1	1A2	1A3	1B1	1C1	1E1	2A1	2B1a	2B1b	ARSC	ARSD	Dβ	ARSE	Actin
Skin-scalp	M	+	− (+)	+	−	−	+	−	+++	+	+	+	−	−	+++
Skin-breast	F	+	− (+)	++	−	−	−	−	++	+/−	+	+/−	−	−	+++
Skin-abdomen	M	++	− (+)	++	−	−	+	−	+++	++	+	+	−	−	+++
Skin-foreskin	M	+	− (+)	+	−	−	+	−	++	−	+	+/−	+	−	+++
Skin-vulva	F	++	− (+)	++	−	−	+/−	−	+++	−	+	+	+	−	+++
Vagina	F	++	− (+)	++	+	−	++	−	+	+++	+	+	+	−	+++
Oral mucosa	F	+	− (+)	+++	−	−	+/−	−	+	+++	+	+	+	−	+++
Esophagus	F	+	− (+)	+	+/−	−	−	−	+	+	++	+	−	−	+++
Stomach	M	+	− (+)	+	+++	+	−	−	+	+/−	+	+	+	−	+++
Duodenum	M	+	+ (+)	+	+++	−	+	+	+	+/−	+	+	+	−	+++
Colon	M	++	− (+)	++	++	−	+	−	+	+/−	++	+	+/−	−	+++
Colorectal	M	+++	+	++	+++	+	+	−	−	+++	++++	++++	+	−	+++
Adrenal	F	+	−	+		−	−	++							+++
Kidney	F	+++	+ (+)	++	++	++	−	−	−	−	++++	+++	+	+	+++
Lung	M	+	+ (+)	++	+	−	+	−	+	+	++++	+	+	−	+++
Trachea	M	+	− (+)	+	−	−	−	−	+	+	+	−	+	−	+++
Prostate	M	+	− (+)	++	−	−	+	−	+	+++	++	+++	+	−	+++
Liver 1	M	++	+	++	+++	+	++	+++							+++
Liver 2	M								−	−	++	+++	−	+	+++
Ovary	F	++	+ (+)	+++	+++	+	+	+	−	−	++	++	+/−	−	+++
Total brain	M	++	+ (+)	++	++	−	+	−	−	−	++	++	−	+	+++
Cerebellum	M	+++	+ (+)	+++	+++	+	+	−	−	−	++	++	−	+	+++
Cerebral cortex	M	++	+ (+)	+	+	−	++	−	−	−	++	++	−	+	+++
Frontal lobe	M	+++	+ (+)	+++	++	+	+	−	−	−	+++	++	−	+	+++

Note. The presence of unspliced SULT 1A2 is indicated in parentheses. Analyses were performed on samples of 0.5 μg total RNA or 0.1 μg mRNA.

manner than is indicated by our results. At least two possible explanations may account for this apparent difference. The presence of PST mRNAs may not correlate with ribosomal translation into functional proteins, possibly due to posttranscriptional and/or posttranslational effects. It is more likely, however, that widespread detection of the two mRNAs by RT-PCR is due to the high sensitivity of this method in comparison with methods of detection at the protein level. The extensive expression of M-PST, if present at the protein level, may be important in delimiting the autocrine/paracrine effects of the ubiquitous catecholamines, while the extensive expression of P-PST, if present at the protein level, may provide a general chemical defense role due to its ability to efficiently sulfate a broad range of substances.

Preliminary data suggested that a portion of the *SULT 1A2* RNA is incorrectly spliced. The unexpected and frequent finding of an amplimer of approximately 430 bp, generally alone, or less often in combination with the anticipated spliced amplimer of 307 bp, in most *SULT 1A2* reactions, suggested the presence of an intron within the longer RNA species. DNA sequencing of the RT-PCR amplimer verified this assumption. We speculate that the *SULT 1A2* gene represents a possible gene duplication of *SULT 1A1* that has undergone mutations rendering it an ineffective or quasi-effective pseudogene, albeit expressed occasionally as RNA (1, 2). When expressed as a recombinant enzyme *in vitro,* the activity is 10–100 fold less active than the *SULT 1A1* gene product (P-PST1) on planar phenolic substrates. The reason for this inaccurate splicing is not clear as the 5′-GU . . . AG-3′ rule is evident. Perhaps sequences internal to the intron affect splicing efficiency.

SULT1B1 appeared to have widespread expression except among skin samples from various sites. The role of the *SULT1B1* gene product to sulfonate thyroid hormone and target it for irreversible inactivation via deiodination is important in maintaining thyroid hormone levels within relatively narrow limits. Since this hormone is involved in regulating the metabolic processes of most tissues, it is not surprising that *SULT1B1* displays extensive expression. *SULT1E1,* most commonly identified with estrogen sulfonation, was also found in most tissues. However, unlike *SULT1B1,* it was present in most skin samples. Many of these tissues are generally known to be estrogen-responsive. Other tissues, such as those of the GI tract are exposed to environmental estrogens where local inactivation by sulfonation may prevent undesired systemic effects.

TABLE IV

RT-PCR Profiles of SULT and ARS Gene Expression in Human Normal (N) and Transformed (T) Cell Cultures: (A) Keratinocytes and Carcinomas, (B) Melanocytes and Melanomas, and (C) Fibroblasts and Fibrosarcomas

		Sulfotransferase								Sulfatase				
Cell line	N/T	1A1	1A2	1A3	1B1	1C1	1E1	2A1	2B1a	2B1b	ARSC	ARSD	ARSDβ	ARSE	β-Actin
							A								
NHEK adult[a]	N	++	−(+)	++	+	−	+	−	+	+++	+++	+++	+	−	+++
NHEK neonatal[a]	N	++	++(+)	++	++	++	+	−	+	+++	+	++	+	−	+++
SCC 9[a]	T	+	−(+)	++	+	−	+	−	+	++	++	++	+	+	+++
SCC 12[a]	T	+	−	++	−	−	−	−	+	++	+++	+++	−	−	+++
SCC 13[a]	T	+	−(+)	++	++	−	+	−	+	+++	++	++	+	+	+++
SCC 15[a]	T	++	+(+)	++	+++	−	+	−	+	++	++	++	++	+	+++
							B								
NHEM[b]	N	+	−(+)	++	++	++	−	+/−	+	−	++	++	+	−	+++
MS7[b]	T	+	−	++	−	+/−	−	−	−	−	++	+	+	+	+++
SK Mel 28[b]	T	+	−(+)	++	++	++	−	+	−	−	+	+	+	−	+++
							C								
HSF[c]	N	+	−	+	+	+	−	−	+	+	++	++	+	−	+++
HT 1080[c]	T	++	+(+)	++	++	−	−	−	−	−	+	+	+	+	+++

[a] Samples represent 0.1 μg of mRNA.
[b] MS7 cells represent 0.1 μg of mRNA. NHEM and SK Mel 28 cells represent 0.5 μg total RNA.
[c] Samples represent an unknown quantity of mRNA. The presence of unspliced SULT 1A2 is indicated in parentheses.

The spatial expression of the steroid sulfating isozymes *SULT 2A1* and *2B1a/b* appears to be more limited than that of the other profiled sulfotransferases, both *in vivo* and in cultured cells with *SULT 2A1* demonstrating the most restricted pattern of expression. This may reflect the limited distribution of hydroxysteroid hormone-producing and -responsive tissues. The involvement of these isozymes in regulating hormone bioavailability implies their association with hormonally influenced developmental processes. A high level of expression of *SULT 2B1a* and *b* in the placenta has suggested a role in fetoplacental unit functioning during pregnancy (55). Little information is currently available about the endogenous substrates of the *SULT2B* isoform products.

The survey of arylsulfatases in tissues indicates the extensive presence of *ARSC* and *D. ARSC* hydrolyzes sulfate esters of a wide range of steroids and cholesterol, and possibly xenobiotic phenols. Endogenous substrates of *ARSD* have not been identified. Although *ARSC* is known to play an important role in restricting steroid activity to target organs, the widespread expression of *ARSC* and *D* indicates extensive involvement in multiple processes or a housekeeping type function. The limited expression of *ARSDβ* and highly restricted expression of *ARSE* may indicate unique roles for these sulfatases.

The expression patterns of cultured keratinocytes and samples of skin were evaluated for identification of candidate STs responsible for cholesterol sulfation, since cholesterol sulfate is a mediator of differentiation. All of the isoforms profiled in this study appeared to be expressed in cultured normal human epidermal keratinocytes (NHEK) of adult origin except *SULT 1A2*, *1C1*, and *2A1* (Table IV). *SULT 2A1* was not expressed in either adult or neonatal NHEK cell lines. There was evidence of *SULT 2A1* in melanocytic cells but it was not detected in five samples of skin obtained from different sites. Therefore, the leading *SULT* candidates responsible for cholesterol sulfation in skin appear to be *SULT 1E1* (encoding estrogen ST) and *SULT 2B1a/b*. Both *SULT 2B1a/b* isoforms are capable of sulfating the prototypic hydroxysteroid DHEA. Although structural similarities between cholesterol and the steroid hormones implicate the involvement of *SULT 1E1* or *2B1a/b*, the possibility remains that one of the phenol sulfotransferases, *SULT 1A1* (P-PST1) or *1A3* (M-PST), may be involved in cholesterol sulfation. *SULT 1A1* and *1A3* continue to be the leading candidates for sulfation of minoxidil in the skin. Enzymatic profiling of each isotype with various substrates and/or immunolocalization is required to resolve these issues.

We had presumed that the patterns of expression of *SULTs* and *ARSs* would be similar for a given tissue and cells derived from that tissue when grown in culture. In general, this premise has been confirmed. However, our survey has demonstrated some exceptions to this rule. For example, *SULT 1B1* and *1C1*

TABLE V

RT-PCR Profiles of SULT 1A1 and 1A3 Distal and Proximal Promoter Use in (A) Human Tissues and (B) Human Normal (N) and Transformed (T) Cell Lines

A

		1A1		1A3		
Normal tissues	Sex	Distal	Prox	Distal	Prox	β-Actin
Skin-scalp	M	−	+	++	+	+++
Skin-breast	F	−	+	+	+	+++
Skin-abdomen	M	+	−	++	+	+++
Skin-foreskin	M	+	−	++	+	+++
Skin-vulva	F	+	−	++	+++	+++
Vagina	F	+	−	+++	++	+++
Oral mucosa	F	+/−	−	++	++	+++
Esophagus	F	+	−	+	+	+++
Stomach	M	+	−	+/−	+/−	+++
Duodenum	M	+	+	+++	+++	+++
Colon	M	+	−	+	+	+++
Colorectal	M	+	+	+	+	+++
Kidney	F	++	+	++	+	+++
Lung	M	+	−	++	+	+++
Trachea	M	−	+	+	+	+++
Prostate	M	+	−	+++	++	+++
Ovary	F	+	−	+	+	+++
Liver	M	+	−	+	+	+++
Total brain	M	+	+	++	+	+++
Cerebellum	M	+	+	++	+	+++
Cerebral cortex	M	++	+	++	+	+++
Frontal lobe	M	+++	+	++	+	+++

B

		1A1		1A3		
Cell line	Norm/tran	Distal	Prox	Distal	Prox	Actin
NHEK adult	N	+	+	+	+	+++
NHEK neonatal	N	+	+	+	+	+++
SCC 9	T	+	+	++	++	+++
SCC 12	T	+	−	+	+	+++
SCC 13	T	+	+	++	+	+++
SCC 15	T	+	+	+	+	+++
NHEM	N	+	+	+	+	+++
MS7	T	+	+	++	++	+++
SK Mel 28	T	+	+/−	++	+	+++
HSF	N	+	+	++	++	+++
HT 1080	T	+	−	++	+	+++

were absent in five skin samples of different origins but appeared to be present in some normal keratinocyte, melanocyte and fibroblast cell lines as well as some of the corresponding transformed cell lines. Differences were also noted with regard to previous investigations conducted at the protein level. Whereas immunohistochemical methods failed to detect PST isozymes in fibroblasts (non-epithelial cells) *in vivo,* we detected the mRNA for several PSTs in normal fibroblast cultures (HSF) and a transformed fibrosarcoma cell line (HT1080). Although these findings may reflect differences in detection sensitivities between the methods, it may also reflect artifactual upregulation of certain enzymes by specific artificial *in vitro* monolayer culture conditions. Changes in expression that occur as a result of culture conditions may have a significant effect on the interpretation of *in vitro* pharmacologic drug and cytotoxicity screens based on cell lines that are capable of metabolizing the test agents. Isozyme profiles would provide general information relevant to questions of: (i) the bioavailability of therapeutic drugs *in vivo;* (ii) the identification of appropriate cell lines

and tissue culture models for the development of *in vitro* pharmacologic assays, and (iii) the manifestation of inverse activity of some potential therapeutic compounds when tested *in vitro* vs *in vivo*.

In general, the patterns of expression of the *SULT* and *ARS* genes in the transformed (cancer) cell lines reflect patterns in the corresponding normal cell lines. An exception was the presence of *ARSE* in the majority of the transformed cell lines and its absence in the corresponding normal cell lines. The appearance of *ARSE* in five of the seven transformed cell lines may indicate a role for this sulfatase in the transformation process.

Heterogeneity in the 5′ untranslated regions (UTR) of cDNAs for members of the PST genes has suggested the presence of at least two separate cis-acting promoter sequences. Tissue specific promoter usage has been supported by experimental findings. As an initial step to understanding the regulation of gene expression, we were interested in determining promoter use of the *SULT1A1* and *1A3* genes in skin and skin-derived cells in culture due to their probable role in bioactivation of minoxidil and possible role in sulfation of cholesterol. As indicated in Table V, there was site-specific *SULT 1A1* promoter usage in skin tissue, whereas both putative distal and proximal promoters generally appear to be functioning in cultured normal and transformed cells of skin origin. Tissue specific promoter usage was seen in the majority of tissues tested, however there was evidence that both promoters were functional in several tissues, particularly those of neural origin. Evidence of promoter activity at both putative distal and proximal sites of *SULT 1A3* was manifest in both skin tissues and cultured cells of skin origin. Utilization of two promoters suggests diverse modes of regulation are operative in the expression of *SULT 1A3* within a given tissue.

We anticipate that future comparisons of the cytosolic *SULT* gene expression profiles for tumor cells with the profiles of the corresponding normal cells will be helpful to oncologists and pharmacologists. A limited amount of reports have been published to date on the expression of *SULT* isozymes within selected cancer histiotypes vs. normal epithelia. An example includes selected human breast cancer cell lines (e.g., MCF-7), which were tested biochemically for P-PST, M-PST, EST and DHEA-ST enzymatic activity (40). Only EST is detectable in normal breast epithelia homogenates, however, the carcinomas displayed high levels of expression of the PSTs and only trace amounts of EST and DHEA-ST activity. Coughtrie and coworkers have demonstrated by immunohistochemistry that STD (DHEA-ST) is expressed in human ductal-type mammary carcinomas, but is lacking in normal breast epithelia (57). All other major cancer histiotypes remain to be screened for *SULT* expression patterns.

Expressed *SULTs* may account for intratumoral bioinactivation of anti-cancer therapeutic molecules, leading to their excretion and thus abrogating their effects. If a selected chemotherapeutic agent is bioinactivated within the tumor cells by sulfonation, then the *SULT* gene(s) expressed by the cells may account for increased resistance to the therapeutic compound independent of conventional drug resistance mechanisms (i.e., multidrug resistance proteins—MDR1, MRP, etc.).

Hormonally regulated epithelial tissues are dependent on, for example, androgens (e.g., prostatic epithelia) or estrogens (e.g., breast epithelia) for maintaining the homeostasis of differentiation vs. proliferation. It is possible that induction of *SULT* expression may convert a hormone-sensitive epithelial tissue (normal or malignant) into a hormone-insensitive tumor by inactivating the hormone. These epithelia adapt to the lack of functioning hormone by activating alternate pathways and thus would be predicted to grow well in the absence of hormone. Therapy-refractory and hormone-insensitive prostate and breast cancers have been well recognized by the oncology community, and pose a significant problem in terms of therapy and prognosis. Expression studies may provide insight into the possibility of bioinactivation of hormones and therapeutic molecules (e.g., anti-androgens or anti-estrogens) within tumors.

Sulfotransferases can contribute to the activation or inactivation of carcinogens or mutagens via sulfonation, which might be implicated in the chemical carcinogenesis process *per se* within the relevant epithelia. Therefore, expression of the *SULT* enzymes within normal epithelia exposed to chemical carcinogens or procarcinogens is likely to contribute to the mechanisms and incidence of tumorigenesis, both positively and negatively, depending on the substrate recognition characteristics of the compounds and on their mutagenic and carcinogenic potential.

To shed further light on this RT-PCR profiling experiment reported here, future studies will focus on improved immunodetection techniques to determine the presence of the *SULTs* at the protein level in human tissue samples and cultured cells.

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REFERENCES

1. Dooley, T. P. (1998a) Cloning of the human phenol sulfotransferase gene family: Three genes implicated in the metabolism of catecholamines, thyroid hormones, and drugs. *Chemico-Biol. Interact.* **109,** 29–41.
2. Dooley, T. P. (1998b) Molecular biology of the human phenol sulfotransferase gene family. *J. Exp. Zool.* **282,** 223–230.
3. Falany, C. N. (1997) Enzymology of human cytosolic sulfotransferases. *FASEB J.* **11,** 206–216.
4. Coughtrie, M. W. H., Bamforth, K. J., Sharp, S., Jones, A. L., Borthwick, E. B., Barker, E. V., Roberts, R. C., Hume, R., and Burchell, A. (1994) Sulfation of endogenous compounds and xenobiotics—Interactions and function in health and disease. *Chemico-Biol. Interact.* **92,** 247–256.
5. Coughtrie, M. W. H. (1996) Sulphation catalysed by the human cytosolic sulphotransferases—Chemical defence or molecular terrorism? *Human Exp. Toxicol.* **15,** 547–555.
6. Her, C., Kaur, G. P., Athwal, R. S., and Weinshilboum, R. M. (1997) Human sulfotransferase SULT1C1: cDNA cloning, tissue-specific expression, and chromosomal localization. *Genomics* **41,** 467–470, doi:10.1006/geno.1997.4683.
7. Rikke, B. A., and Roy, A. K. (1996) Structural relationships among members of the mammalian sulfotransferase gene family. *Biochim. Biophys. Acta* **1307,** 331–338.
8. Wang, J., Falany, J. L., Falany, C. N. (1998) Expression and characterization of a novel thyroid hormone-sulfating form of cytosolic sulfotransferase from human liver. *Mol. Pharmacol.* **53,** 274–82.
9. Weinshilboum, R. M., Otterness, D. M., Aksoy, I. A., Wood, T. C., Her, C., and Raftogianis, R. B. (1997) Sulfotransferase molecular biology: cDNAs and genes. *FASEB J.* **11,** 3–14.
10. Campbell, N. R. C., Van Loon, J. A., and Weinshilboum, R. M. (1987) Human liver phenol sulfotransferase: Assay conditions, biochemical properties and partial purification of isozymes of the thermostable form. *Biochem. Pharmacol.* **36,** 1435–1446.
11. Falany, C. N., Vasquez, M. E., Heroux, J. A., and Roth, J. A. (1990) Purification and characterization of human liver phenol-sulfating phenol sulfotransferase. *Arch. Biochem. Biophys.* **278,** 312–318
12. Falany, C. N. (1991) Molecular enzymology of human liver cytosolic sulfotransferases. *Trends Pharmacol. Sci.* **2,** 255–259.
13. Goldstein, D. S., Lenders, J. W. M., Kaler, S. G., and Eisenhofer, G. (1996) Catecholamine phenotyping: Clues to the diagnosis, treatment, and pathophysiology of neurogenetic disorders. *J. Neurochem.* **67,** 1781–1790.
14. Reiter, C., and Weinshilboum, R. (1982) Platelet phenol sulfotransferase activity: Correlation with sulfate conjugation of acetaminophen in man. *Clin. Pharmacol. Ther.* **32,** 612–621.
15. Sundaram, R. S., Szumlanski, C., Otterness, D., Van Loon, J. A., and Weinshilboum, R. M. (1989) Human intestinal phenol sulfotransferase: Assay conditions, activity levels and partial purification of the thermolabile (TL) form. *Drug Metab. Disp.* **17,** 255–264.
16. Visser, T. J. (1994) Role of sulfation in thyroid hormone metabolism. *Chemico-Biol. Interact.* **92,** 293–303.
17. Visser, T. J. (1996) Role of sulfate in thyroid hormone sulfation. *Eur. J. Endocrinol.* **134,** 12–14.
18. Zou, J., Pentney, R., and Roth, J. A. (1990) Immunohistochemical detection of phenol sulfotransferase-containing neurons in human brain. *J. Neurochem.* **55,** 1154–1158.
19. Price, R. A., Spielman, R. S., Lucena, A. L., Van Loon, J. A., Maidak, B. L., and Weinshilboum, R. M. (1989) Genetic polymorphism for human platelet thermostable phenol sulfotransferase (TS PST) activity. *Genetics* **122,** 905–914.
20. Eisenhofer, G., Coughtrie, M. W., and Goldstein, D. S. (1999) Dopamine sulphate: An enigma resolved. *Clin. Exp. Pharmacol. Physiol. Suppl.* **26,** S41–S53.
21. Buhl, A. E., Waldon, D. J., Baker, C. A., and Johnson, G. A. (1990) Minoxidil sulfate is the active metabolite that stimulates hair follicles. *J. Invest. Dermatol.* **95,** 553–557.
22. Dooley, T. P., Walker, C. J., Hirshey, S. J., Falany, C. N., and Diani, A. R. (1991) Localization of minoxidil sulfotransferase in rat liver and the outer root sheath of anagen pelage and vibrissa follicles. *J. Invest. Dermatol.* **96,** 65–70.
23. Raftogianis, R. B., Wood, T. C., Otterness, D. M., Van Loon, J. A., and Weinshilboum, R. M. (1997) Phenol sulfotransferase pharmacogenetics in humans: Association of common SULT1A1 alleles with TS PST phenotype. *Biochem. Biophys. Res. Commun.* **239,** 298–304, doi:10.1006/bbrc.1997.7466.
24. Anderson, R. J., Kudlacek, P. E., Clemens, D. L. (1998) Sulfation of minoxidil by multiple human cytosolic sulfotransferases. *Chemico-Biol. Interact.* **109,** 53–67.
25. Hume, R., and Coughtrie, M. W. H. (1994) Phenolsulphotransferase: Localization in kidney during human embryonic and fetal development. *Histochem. J.* **26,** 850–855.
26. Hume, R., Barker, E. V., and Coughtrie, M. W. H. (1996) Differential expression and immunohistochemical localization of the phenol and hydroxysteroid sulphotransferase enzyme families in the developing lung. *Histochem. Cell Biol.* **105,** 147–152.
27. Windmill, K. F., Christiansen, A., Teusner, J. T., Bhasker, C. R., Birkett, D. J., Zhu, X., and McManus, M. E. (1998) Localisation of aryl sulfotransferase expression in human tissues using hybridisation histochemistry and immunohistochemistry. *Chemico-Biol. Interact.* **109,** 341–346.
28. Dooley, T. P., Obermoeller, R. D., Leiter, E. H., Chapman, H. D., Falany, C. N., Deng, Z., and Siciliano, M. J. (1993) Mapping of the phenol sulfotransferase gene (*STP*) to human chromosome 16p12.1-p11.2 and to mouse chromosome 7. *Genomics* **18,** 440–443, doi:10.1006/geno.1993. 1494.
29. Dooley, T. P., Mitchison, H. M., Munroe, P. B., Probst, P. Neal, M., Siciliano, M. J., Deng, Z., Doggett, N. A., Callen, D. F., Gardiner, R. M., and Mole, S. E. (1994a) Mapping of two phenol sulfotransferase genes, STP and STM, to 16p: Candidate genes for Batten Disease. *Biochem. Biophys. Res. Commun.* **205,** 482–489, doi:10.1006/bbrc.1994.2691.
30. Dooley, T. P., and Huang, Z. (1996) Genomic organization and DNA sequences of two human phenol sulfotransferase gene (STP1 and STP2) on the short arm of chromosome 16. *Biochem. Biophys. Res. Commun.* **228,** 134–140, doi:10.1006/bbrc.1996.1628.
31. Ozawa, S., Nagata, K., Shimada, M., Ueda, M., Tsuzuki, T., Yamazoe, Y., and Kato, R. (1995) Primary structures and properties of two related forms of aryl sulfotransferases in human liver. *Pharmacogenetics* **5** (Supp.), 135–140.
32. Zhu, X., Veronese, M. E., Iocco, P., and McManus, M. E. (1996) cDNA cloning and expression of a new form of human aryl sulfotransferase. *Int. J. Biochem. Cell Biol.* **28,** 565–571.
33. Bernier, F., Lopez-Solache, I., Labrie, F., and Luu-The, V. (1994) Cloning and expression of cDNA encoding human placental estrogen sulfotransferase. *Mol. Cell. Endocrinol.* **99,** R11–R15.
34. Jones, A. L., Hagen, M., Coughtrie, M. W. H., Roberts, R. C., and Glatt, H. (1995) Human platelet PSTs: cDNA cloning, stable expression in V79 cells and identification of a novel allelic variant of the phenol-sulfating form. *Biochem. Biophys. Res. Commun.* **208,** 855–862, doi:10.1006/bbrc. 1995.1414.
35. Wilborn, T. W., Comer, K. A., Dooley, T. P., Reardon, I. M., Heinrikson, R. L., and Falany, C. N. (1993) Sequence analysis

and expression of the cDNA for the phenol-sulfating form of human liver PST. *Mol. Pharmacol.* **43,** 70–77.

36. Zhu, X., Veronese, M. E., Bernard, C. C. A., Sansom, L. H., and McManus, M. E. (1993a) Identification of two human brain aryl sulfotransferase cDNAs. *Biochem. Biophys. Res. Commun.* **195,** 120–127, doi:10.1006/bbrc.1993.2018.
37. Zhu, X., Veronese, M. E., Bernard, C. C. A., Sansom, L. H., and McManus, M. E. (1993b) Molecular characterization of a human aryl sulfotransferase cDNA. *Biochem. Biophys. Res. Commun.* **192,** 671–676, doi:10.1006/bbrc.1993.1467.
38. Barker, E. V., Hume, R., Hallas, A., and Coughtrie, M. W. H. (1994) Dehydroepiandrosterone sulfotransferase in the developing human fetus: Quantitative biochemical and immunological characterization of the hepatic, renal, and adrenal enzymes. *Endrocrinology* **134,** 982–989.
39. Falany, C. N., Comer, K. A., Dooley, T. P., and Glatt, H. (1995) Human dehydroepiandrosterone sulfotransferase: Purification, molecular cloning, and characterization. *Ann. N.Y. Acad. Sci.* **774,** 59–72.
40. Falany, J. L., and Falany, C. N. (1996) Expression of cytosolic sulfotransferases in normal mammary epithelial cells and breast cancer cell lines. *Cancer Res.* **56,** 1551–1555.
41. Jedlitschky, G., Leier, I., Buchholz, K., Kurz, G., and Keppler, D. (1996) Transport of glutathione, glucuronate, and sulfate conjugates by the MRP gene-encoded conjugate export pump. *Cancer Res.* **56,** 988–994.
42. Elias, P. M., Williams, M. L., Maloney, M. E., Bonifas, J. A., Brown, B. E., Grayson, S., and Epstein, E. H., Jr. (1984) Stratum corneum lipids in disorders of cornification. *J. Clin. Invest.* **74,** 1414–1421.
43. Jetten, A. M., George, M. A., Nervi, C., Boone, L. R., and Rearick, J. I. (1989) Increased cholesterol sulfate and cholesterol sulfotransferase activity in relation to the multi-step process of differentiation in human epidermal keratinocytes. *J. Invest. Dermatol.* **92,** 203–209.
44. Chida, K., Sagara, H., Suzuki, Y., Murakami, A., Osada, S., Ohno, S., Hirosawa, K., and Kuroki, T. (1994) The beta isoform of protein kinase C is localized on rough endoplasmic reticulum. *Mol. Cell. Biol.* **14,** 3782–3790.
45. Denning, M. F., Kazanietz, M. G., Blumberg, P. M., and Yuspa, S. H. (1995) Cholesterol sulfate activates multiple protein kinase C isoenzymes and induces granular cell differentiation in cultured murine keratinocytes. *Cell Growth Diff.* **6,** 1619–1626.
46. Ikuta, T., Chida, K., Tajima, O., Matsuura, Y., Iwamori, M., Ueda, Y., Mizuno, K, Ohno, S., and Kuroki, T. (1994) Cholesterol sulfate, a novel activator of the eta isoform of protein kinase C. *Cell Growth Diff.* **5,** 943–947.
47. Osada, S., Mizuno, K., Saido, T., Akita, Y., Suzuki, K., Kuroki, T., and Ohno, S. (1990) A phorbol ester receptor/protein kinase, $nPKC_{eta}$, a new member of the protein kinase C family predominantly expressed in lung and skin. *J. Biol. Chem.* **265,** 22434–22440.
48. Osada, S., Hashimoto, Y., Nomura, S., Kohno, Y., Chida, K., Tajima, O., Kubo, K., Akimoto, K., Koizumi, H., Kitamura, Y., Suzuki, K., Ohno, S., and Kuroki, T. (1993) Predominant expression of $nPKC_{eta}$, a Ca^{2+}-independent isoform of protein kinase C in epithelial tissues, in association with epithelial differentiation. *Cell Growth Diff.* **4,** 167–175.
49. Ueda, E., Ohno, S., Kuroki, T., Livneh, E., Yamada, K., Yamanishi, K., and Yasuno, H. (1996) The eta isoform of protein kinase C mediates transcriptional activation of the human transglutaminase 1 gene. *J. Biol. Chem.* **271,** 9790–9794.
50. Ballabio, A., Parenti, G., Carrozzo, R., Sebastio, G., Andria, G., Buckle, V., Fraser, N., Craig, I., Rocchi, M., Romeo, G., *et al.* (1987) Isolation and characterization of a steroid sulfatase cDNA clone: Genomic deletions in patients with X chromosome-linked ichthyosis. *Proc. Natl. Acad. Sci. USA* **84,** 4519–4523.
51. Franco, B., Meroni, G., Parenti, G., Levilliers, J., Bernard, L., Gebbia, M., Cox, L., Maroteaux, P., Sheffield, L., Rappold, G. A., Andria, G., Petit, C., and Ballabio, A. (1995) A cluster of sulfatase genes on Xp22.3: Mutations in chondrodysplasia punctata (CDPX) and implications for warfarin embryopathy. *Cell* **81,** 15–25.
52. Yen, P. H., Allen, E., Marsh, B., Mohandas, T., Wang, N., Taggart, R. T., and Shapiro, L. J. (1987) Cloning and expression of steroid sulfatase cDNA and the frequent occurrence of deletions in STS deficiency: Implications for X-Y interchange. *Cell* **49,** 443–454.
53. Fujita, K., Nagata, K., Ozawa, S., Sasano, H., and Yamazoe, Y. (1997) Molecular cloning and characterization of rat ST1B1 and human ST1B2 cDNAs, encoding thyroid hormone sulfotransferases. *J. Biol. Chem.* **122,** 1052–1061.
54. Sakakibara, Y., Katafuchi, J., Ringer, D. P., Takami, Y., Nakayama, T., Suiko, M., and Liu, M. C. (1998) Molecular cloning, expression, and characterization of novel human SULT1C1 sulfotransferases that catalyze the sulfonation of N-hydroxy-2-acetyl-aminofluorene. *J. Biol. Chem.* **273,** 33929–33935.
55. Her, C., Wood, T. C., Eichler, E. E., Mohrenweiser, H. W., Ramagli, L. S., Siciliano M. J., and Weinshilboum, R. M. (1998) Human hydroxysteroid sulfotransferase SULT2B1: Two enzymes encoded by a single chromosome 19 gene. *Genomics* **53,** 284–295, doi:10.1006/geno.1998.5518.
56. Sambrook, J., Maniatis, T., and Frisch, E. F. (1989) Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, New York.
57. Sharp, S., Anderson, J. M., and Coughtrie, M. W. H. (1994) Immunohistochemical localisation of hydroxysteroid sulphotransferase in human breast carcinoma tissue: A preliminary study. *Eur. J. Cancer* **30A,** 1654–1659.