Altered tissue distribution of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine-DNA adducts in mice transgenic for human sulfotransferases 1A1 and 1A2

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Abbreviations: PAC, P1-derived artificial chromosome; PCR, polymerase chain reaction; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine; SULT, soluble sulfotransferase.

Introduction

Soluble sulfotransferases (SULTs) transfer the negatively charged sulfonate group from 3'-phosphoadenosine-5'-phosphosulfate to nucleophilic sites of small endogenous compounds and xenobiotics (1,2). Due to their charge, the resulting conjugates do not passively permeate cell membranes, which facilitates their vectorial transport and excretion. However, the sulfate moiety, resulting from O-sulfonation, is a good leaving-group in certain chemical linkages—hence, the respective conjugates are electrophilically reactive (2). For example, 2-acetylaminofluorene—the first carcinogen whose activation mechanism was elucidated—is terminaly activated by sulfonation (reviewed in 3). We have expressed various human and rodent SULTs in bacterial and mammalian target cells of standard in vitro mutagenicity tests (4). Using these models, we demonstrated the activation of >100 substances to mutagens by SULT activity. This list comprises plant metabolites (alkenylbenzenes, glucosinolates), heat-induced food constituents (hydroxymethyl-substituted furans, heterocyclic amines), environmental contaminants (alkylated polycyclic hydrocarbons) and drugs (tamoxifen, hyancantine, phenacetin). Thus, sulfocojugation is a rather common activation mechanism.

The structure of the SULT superfamily and the tissue distribution and substrate specificity of SULTs show pronounced differences between species. For example, a single SULT2A form occurs in humans, whereas at least three forms exist in mice and rats (5). Rat (r) SULT 2A3 efficiently activates a-hydroxymatroxin to a genotoxicant, an activity not seen with the remaining rSULT2A forms or any human SULTs (6,7). rSULT2A3 is expressed primarily in liver and is pivotal in the strong hepatocarcinogenicity of tamoxifen, an anti-breast cancer drug, in this species. Another subfamily, SULT1A, has selectively expanded in primates. Four genes belonging to this subfamily have been identified in humans, whereas a single SULT1A gene was detected in all non-primate mammalian species studied (8). The members of the human (h) SULT1A subfamily have functionally diversified. Whereas catecholamines are the principal substrates for hSULT1A3 and 1A4 (which are identical on the protein level), hSULT1A1 and 1A2 have adopted unusually broad substrate specificity toward xenobiotics. Promutagens activated by hSULT1A1 range from small molecules (such as 2-nitropropane) to large molecules (such as 6-hydroxymethylanthranthrene) (9). Moreover, hSULT1A1 protein, unlike rodent SULT1A1, is found at high levels in many tissues. For example, it reaches nearly the same high level in ileum as in the liver (10). In contrast, in rats, SULT1A1 was not detected in the intestine (11).

hSULT1A2, resulting from a recent gene duplication, has been localized to 16p12.1–11.2. ~8500 bp downstream of hSULT1A1 (5,12). hSULT1A1 and 1A2 show strongly overlapping substrate specificities but often vary in their kinetic parameters. N-Hydroxy-2-acetylaminofluorene is an example of a promutagen that is more efficiently activated by hSULT1A2 than by hSULT1A1 (13). However, the level of hSULT1A2 protein is lower than that of hSULT1A1 in all tissues examined (10,14).

2-Amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) (15) is the most abundant heterocyclic aromatic amine found in fried meat and fish (16). In rats, it induces tumors in colon, mammary gland and prostate (17,18). These are common tumors in countries with Western lifestyle. Since only few chemicals are known to induce tumors at these sites in animal models, PhIP has raised much interest. However, the target tissues of PhIP vary between laboratory animal species and are unknown in humans. In mice, PhIP primarily induces lymphoid tissue tumors (19) and small intestinal adenocarcinomas (20).

PhIP itself is non-genotoxic. The proximate genotoxicant, N'-OH-PhIP, is formed by cytochromes P450 (CYPs), with CYP1A2 being particularly active (21). Subsequently, acetyltransferases (NATs) or SULTs form reactive esters (22,23). In mutagenicity assays with Salmonella strains lacking the endogenous acetyltransferase but expressing various human enzymes, N'-OH-PhIP was activated specifically by hSULT1A1 and hSULT1A2 but not by hNAT1 or hNAT2 (15,24). Likewise, PhIP was strongly mutagenic in Chinese hamster...
V79 cells engineered for coexpression of hCYPIA2 and hSULT1A1, but only weakly in cells coexpressing hCYPIA2 and hNAT2 and not at all in cells expressing hCYPIA2 alone or in combination with hNAT1 (15). These findings suggest that hSULT1A1 plays a prominent role in the activation of PhIP. Rat and mouse SULT1A1 did not activate N\textsubscript{2}-OH-PhIP to a mutagen in the Salmonella and/or V79 cell systems; however, activation was observed with rNAT1, rSULT1C1 and mouse (m) SULT1D1 (15); none of these enzymes reached the high activation efficiency of hSULT1A1.

In order to investigate the hSULT1A1/IA2-mediated activations in an in vivo experimental model, we constructed mouse lines transgenic for the human SULT1A1/SULT1A2 gene cluster. The transgenic mice were used to study the activation of the model compound PhIP.

Material and methods

Generation of transgenic mice

The P1-derived artificial chromosome (PAC) human DNA library 4 from the Roswell Park Institute (RPCI-4) was screened with a hSULT1A1 complementary DNA probe. The ends of the human DNA inserts of positive clones were sequenced (Agowa, Berlin, Germany) using the T7 and Sp6 promoter primer sequences at the 3'-ends of the vector pCYPA2 (http://bapac.chori.org/pcyapac2.htm). The human genomic insert of the clone selected (7041J18921Q2) was 117.5 kb long. It contained no further genes in the 35 kb region upstream of SULT1A1, but two additional genes—CCDC101 and NUPR1—downstream of SULT1A2. The entire NUPR1 gene (on the same strand as the SULTs) and the first exon of CCDC101 (on the complementary strand) were located between two SalI restriction sites. Digestion of the PAC with SalI (Fermentas, St Leon-Rot, Germany) (37°C, 2 h), heat inactivation of the restriction mixture (65°C, 20 min), religation of the fragments with T4 ligase (8°C, overnight), electroreplication of the product into Escherichia coli and selection on kanamycin-containing lysogeny broth provided colonies harboring a new PAC molecule missing the 48.9 kb fragment downstream of SULT1A2 (Figure 1A).

The single SalI restriction site in the new PAC at the junction of vector and insert was used to linearize the DNA (5 μg, 2 h, 37°C) (Figure 1A). After heat inactivation (65°C, 20 min), the restriction mixture was diluted directly in microinjection buffer (10 mM Tris–HCl pH 7.5, 0.1 mM ethylenediaminetetraacetic acid, 100 mM NaCl, 70 mM spermidine, 30 mM spermine) to a final concentration of 1–2 μg/ml for microinjection into pronuclei of fertilized oocytes of FVB/N mice.

Genotyping

Genomic DNA from tail biopsies was screened for transgenicity by polymerase chain reaction (PCR). Four different regions of the transgene were analyzed (PCR1–4 in Figure 1). Each PCR reaction was conducted in a 50 μl volume containing 20 ng DNA, 1 U Taq polymerase, 10 pmol of each primer, 6.25 mM per diethylnitrophenyl thiophosphate and 75 mM MgCl\textsubscript{2}. PCR was performed in a T-gradient thermocycler (Biometra, Goettingen, Germany): 5 min 94°C (hot start) and 40 cycles at 94, 60 and 72°C for 40, 40 and 30–120 s, respectively. The primer sets (BioTeZ, Berlin, Germany) were: 5'-GACTAAGGCCCCTCATCCATCC-3' and 5'-CCCATTGAGGACCTGAG TCTAG-3' for PCR1 (241 bp product); 5'-GAGCTCAAGGACCAGT GCTGACTAC-3' either together with 5'-GCTCTCAAACCTCCGGCT CAGTTGATCTG-3' for PCR2 (656 bp product) or together with 5'-GGTGAAG TCTCGCTGACTCAAACCTTG-3' for PCR3 (1094 bp product); 5'- CACTTAATCAACTGGGACACCCTCGCCATG-3' and 5'-TCAGCCTCC CAAATGTCGAGGATACAG-3' for PCR 4 (508 bp product). PCR products were visualized by electrophoresis in 1–2% agarose gels using the Gene Ruler DNA Ladder Mix (Fermentas, St Leon-Roth, Germany) as standard.

Determination of transgene copy number

The transgene copy number was determined by quantitative real-time–PCR on an MX3005P Quantitative PCR System (Stratagene, La Jolla, CA). DNA from tail biopsies was analyzed in triplicate for the transgene and the reference gene (β-globin). We amplified two alternative sequences of the transgene (PCR5, 141 bp product; PCR6, 239 bp product; for location and primers see Supplementary Figure 1, available at Carcinogenesis Online). The primers for amplifying a 290 bp β-globin sequence were 5'-AGTTTGTGTTGGTACGGCT GGGCAAGGTGGTGATC-3' and 5'-CTTGAGGCGCCGCTCAAGTGATT CAGGCCATC-3'. On each plate, a standard row for transgene and reference (isolated PCR products in dilutions from 10\textsuperscript{3} to 10\textsuperscript{9} molecules per well) were coamplified. Each reaction was conducted with the ImmoMix kit (Bioline, Luckenwalde, Germany) in a 25 μl volume containing 20 ng DNA, 200 μM of each primer and 1 SYBR green solution. PCR conditions were: 7 min 95°C (hot start) and 36 cycles 94, 60 and 72°C for 30, 25 and 25 s, respectively. Unspecific amplifications were checked by melting curves (95–58°C).

Determination of sites of transgene integration

Blood was withdrawn and lymphocytes were cultured and treated with colcemid as recommended by the Jackson Laboratory (http://www.jax.org/cyto/ blood_preps.html). Fluorescence in situ hybridization was performed using the Spectral Karyotyping (Sky)-Paint-Kit-DNA-M10 (Applied Spectral Imaging, Neckarhausen, Germany) and the hSULT1A1/IA2-PAC DNA as a probe (25,26).

Maintenance and breeding of animals

FVB/N wild-type and transgenic mice were bred by brother and sister mating. They were kept under specific pathogen-free conditions in plastic cages at 22 ± 2°C and 55 ± 5% humidity. They were maintained under a standard 12 h light/12 h dark cycle with water and standard chow (Altromin 1326 pellets; Altromin, Lage, Germany) provided ad libitum.

Fig. 1. PCR used for genotyping hSULT1A1/IA2-transgenic mouse lines. (A) Scheme of the human PAC DNA used for microinjection (85 kb total). SalI restriction site used for linearization. (B) Mouse tail DNA was amplified with primers for PCR1 (241 bp product), PCR2 (656 bp product), PCR3 (1094 bp product) and PCR4 (508 bp product). PCR products were separated on 1.5% agarose gels. tg\textsubscript{1}, tg\textsubscript{2}, tg\textsubscript{3}, mice of different hSULT1A1/IA2-transgenic lines; wt, wild-type mouse; PAC, hSULT1A1/IA2-PAC DNA (positive control); H\textsubscript{2}O, no DNA.
Immunoblot analyses

Eight-week-old animals were used. Intestines were immediately opened, cleansed, placed on an ice-cooled glass plate and rinsed with ice-cold phosphate-buffered saline. The mucosa was scraped off with a plastic scraper. Intestinal mucosa and other tissues were homogenized in three to five volumes of 10 mM sodium phosphate buffer (pH 7.4) containing 150 mM KCl and 0.1% Complete Protease Inhibitor Cocktail (Roche Diagnostics, Mannheim, Germany). Cell debris was removed by centrifugation (15 min, 3000g, 4°C). Cytosolic fractions, obtained as supernatant after ultracentrifugation (60 min, 10 000g, 4°C), were stored at −80°C. The protein content was determined with the bicinchoninic acid protein assay (Pierce, Rockford, IL) using bovine serum albumin as a standard.

Cytosolic fractions were resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (11%, w/v). hSULT1A1, hSULT1A2, mSULT1A1 and mSULT1D1 expressed in Salmonella typhimurium TA1538 were used as standards (14,27). Electrophoresed proteins were electro transferred to Hybond ECL membrane (GE Healthcare, Freiburg, Germany), which was blocked subsequently with bovine serum albumin (1% w/v) dissolved in Tris-buffered saline (10 mM Tris/HCl (pH 7.6), 150 mM NaCl, 0.1% Tween 20) at 25°C for 1 h. Tris-T was used for diluting antisera [1:2000 to 1:10 000 for primary antiserum, 1:2000 for secondary anti-rabbit or anti-sheep antibodies coupled with horseradish peroxidase (Sigma–Aldrich, Taufkirchen, Germany)]. The membranes were probed with antiserum A (28) and B (10), raised in sheep and rabbit, respectively, against hSULT1A forms. Antiserum A detects all hSULT1A forms. Antiserum B detects hSULT1A1 and 1A3, but only shows weak activity with hSULT1A4. Immunoreactive bands were visualized using the enhanced chloramphenol luminescence detection kit (GE Healthcare, Braunschweig, Germany) together with the Fuji LAS-1000 imaging system (Raytest, Straubenhardt, Germany).

4-Nitrophenol sulfation in tissue preparations

Incubations contained cytosolic fractions (1–200 µg protein, linearity of the reactions ensured), the cofactor 3'-phosphoadenosine-5'-phosphosulfate (50 µM), potassium phosphate buffer (50 mM, pH 7.4), MgCl2 (5 mM) and 4-nitrophenol (4 µM) in a final volume of 100 µl. All components except the substrate were warmed to 37°C for 2 min. Then, 4-nitrophenol was added using water (5 µl) as a solvent. The reaction was incubated at 37°C for 5 min and then stopped by heating for 2 min at 95°C and transferred on ice for 10 min. After centrifugation at 23 000g for 15 min, 60 µl of the supernatant was transferred into a high-performance liquid chromatography vial. An aliquot of 20 µl was directly analyzed using a Waters 2960 HPLC system (Waters, Eschborn, Germany). Samples were separated isocratically using 0.1 M KH2PO4, 0.1% methanol in water on a NovaPak C18 150 mm × 3.9 mm column (Waters, Eschborn, Germany). The protein content was determined with the bicinchoninic acid protein assay (Pierce, Rockford, IL) using bovine serum albumin as a standard.

Results

Generation of hSULT1A1/1A2-transgenic mouse lines

The linearized PAC construct (Figure 1A) was microinjected into pronuclei of fertilized FVB/N oocytes. Progeny was analyzed by PCR using four sets of primers for different regions of the human transgenes. Three mice, positive for all PCR probes (Figure 1B), are the founders of three independent transgenic lines, designated tg1, tg2 and tg3.

Chromosomal integration sites

The transgene was visualized by fluorescence in situ hybridization on metaphase chromosomes. Figure 2 (lower panels) depicts examples of a homozygous tg1 animal and hemizygous tg2 and tg3 animals. For the mapping of the fluorescence in situ hybridization signal, the chromosomes were counterstained using chromosome-specific DNA probes labeled with defined fluorophores (spectral karyotyping) (Figure 2, upper panels). A single integration locus per haploid genome was determined for tg1 (chromosome 9, section A5.3) and tg2 (chromosome 9, section F3), whereas two integration loci (chromosome 4, section D2.2–2.3 and chromosome 11, section E1) were observed in tg3.

Transgene copy number

The number of transgene copies in the genome of transgenic mice was determined relative to the β-globin gene in quantitative real-time PCRs. The lowest transgene signal was detected in hemizygous tg2 animals. Line tg1 carried ~10 times as many transgene copies as tg2. A particularly high variation in copy numbers was observed in line tg3 due to independent segregation of the two chromosomes carrying the transgene. After separation of the two chromosomes by backcrossing against wild-type animals, we obtained one line (tg4) with ~2-fold higher and another line (tg5) with 4- to 5-fold higher copy numbers than tg2 (Supplementary Table 1 is available at Carcinogenesis Online). Moreover, after injection of other hSULT constructs (hSULT1B1 or hSULT1A1 variants), we obtained new lines (to be described elsewhere). Since all lines had some regions (either in the vector or the hSULT transgene) in common (Supplementary Figure 1 is available at Carcinogenesis Online), we were able to compare them with respect to transgene copy number. Those hemizygous animals with the lowest relative copy number had lower copy numbers than hemizygous tg2 mice (using β-globin for normalization) and a series of human DNA samples studied (using the DNA amount for normalization). Therefore, we suspect that these lines have one hSULT copy in the hemizygous state. On this basis, hemizygous tg1, tg2, tg4 and tg5 mice appear to have 43, 4, 9 and 19 copies, respectively. In humans, a copy number polymorphism of SULT1A1 has been detected (31). One to approximately five SULT1A1 copies had been detected in 461 subjects (Caucasian-Americans and African-Americans).

Phenotype of tg animals—preliminary data

Expression of transgenes may interfere with host functions and affect the phenotype. However, none of the transgenic SULT lines presented...
Transgenic hSULT1A1/1A2 mice

Fig. 2. Spectral karyotyping (upper panels) and one-color fluorescence in situ hybridization (lower panels) for detection of the transgenes in mouse chromosomes. Transgene integration could be visualized in both chromosomes 9, section A5.3, of a homozygous tg1 mouse (A); in one chromosome 9, section F3, in a hemizygous tg2 mouse (B); and in two different chromosomes—chromosome 4, section D2.2–2.3 and chromosome 11, section E1—in a hemizygous tg3 mouse.

in this paper (as well as other lines listed in Supplementary Table 1, available at Carcinogenesis Online) showed an obvious macroscopic or clinical deviation from wild-type FVB/N mice regarding litter size, distribution of sexes, food and water consumption, growth rate, fertility as well as weights and macroscopic morphology of organs. However, we would have missed any phenotypic changes manifested at higher ages since most animals were killed in experiments at an age of 12–15 weeks.

In order to study possible influences of human SULT transgenes on the hepatic transcriptome, whole genome microarrays (five 8-week-old male animals per group) hemizygous animals of lines tg1 and tg4 (described in this study) and other hSULT lines constructed (tg6, tg11 and tg12, for details on transgenes and copy numbers see Supplementary Table 1, available at Carcinogenesis Online) were compared with the wild-type. The results will be published elsewhere. However, since CYP1A enzymes play an important role in the activation of PhIP, they could influence results in an animal experiment if their expression levels were different between wild-type and transgenic mice. The data provided in Supplementary Table 2, available at Carcinogenesis Online, show that neither Cyp1a1 nor 1a2 messenger RNA levels were altered in the livers of any of the hSULT transgenic lines studied compared with the wild-type. Expression changes of other genes were rare and mild.

Transgene expression

The expression of the hSULT proteins was analyzed by immunoblotting (Figure 3) using anti-hSULT1A antisera that cross-reacted only marginally with mSULTs. Cytosolic preparations of Salmonella expressing human and murine SULT forms served as positive or cross-reactivity controls, respectively. Strong expression of both hSULT1A proteins was observed in liver, kidney, lung and intestine (duodenum, jejunum, ileum, cecum and colon) of line tg1; expression was weaker in stomach, pancreas, heart, brain and testis (Figure 3A). Both human proteins were also observed in spleen and uterus but not in skin and urinary bladder (data not shown). hSULT1A2 was generally present at lower levels than hSULT1A1 and was not detected in brain, heart and testis.

The tissue distribution of the SULT proteins was similar in lines tg1, tg2 and tg3. However, the expression levels differed between the lines, as exemplified for liver and colon in Figure 3B. Densitometric analysis of the immunoblots yielded ~10 times lower SULT1A1 levels in hemizygous tg2 animals than in hemizygous tg1 animals in both tissues. Expression in tg3 was similar to that in tg1. However, when the experiment was conducted, we did not yet know that two integration events had taken place in line tg3. The animal analyzed was a direct descendent from the founder animal bred with a wild-type mouse. Therefore, this particular animal could have had both or either of the two transgenic chromosomes, which could not be specified retrospectively.

In line tg1, we studied the levels of the hSULT proteins in liver, kidney, lung, jejunum and colon in more detail. We conducted immunoblots using several different amounts of the tissue samples as well as standards with known SULT content. Representative blots for each tissue are depicted in the Supplementary Figure 3, available at Carcinogenesis Online. The results are summarized separately for both sexes in Table I. The differences in the hSULT levels between the sexes were minimal. The hSULT1A1 content of cytosolic protein of tg1 mice (mean of five males and five females) amounted to 4.3% in liver, 1.9% in jejunum, 1.2% in kidney, 0.5% in lung and 0.9% in colon. The corresponding values for hSULT1A2 were lower by a factor of 3–6. Table I also contains published data for hSULT1A1 and hSULT1A2 levels in the respective human tissues. Levels were clearly higher in tg1 mice than in humans, in agreement with the high number of gene copies in this transgenic line.

Furthermore, we determined the sulfation activities with the probe substrate 4-nitrophenol in liver, kidney, lung, jejunum and colon from male wild-type and tg1 mice. tg1 animals showed roughly 10-fold higher sulfation activity in each tissue analyzed than the wild-type (Table II).

Levels and tissue distribution of DNA adducts formed by PhIP

PhIP formed higher levels of DNA adducts in tg1 mice than in wild-type mice (Figure 4). The increases in adduct levels were strongest in liver (13-fold), lung (3.8-fold) and colon (2.0-fold). These differences were statistically highly significant (P < 0.001). Smaller, statistically significant increases occurred in kidney (1.6-fold) and cecum (1.5-fold). Although adduct levels in jejunum, ileum and spleen of the transgenics were also higher than in wild-type animals (1.2- to 1.7-fold), these differences were not statistically significant.
Discussion

SULTs from humans and common laboratory animals substantially differ in substrate specificity, tissue distribution and regulation. We have introduced the hSULT1A1/1A2 gene cluster with large flanking regions into mice. We hypothesized that elements in the flanking sequences may confer some human regulatory factors to the transgenic models. This approach had been successful for expressing other human genes encoding xenobiotic-metabolizing enzymes, e.g. CYP2D6 (33) or CYP1A1 and 1A2 (34), in mice.

In humans, SULT1A1 is widely distributed throughout the body, with high abundance in liver, lung, brain, platelets, gastrointestinal
Therefore, other phase-2 enzymes were required for the activation in
highly expressed in liver, did not activate murine hepatic phase-2 enzymes. In particular, mSULT1A1, which is
consistently lower than that of hSULT1A1 (in the range of 15–30% of
hSULT1A1). SULT1A2 is a minor form in humans. Quantifiable sig-
tications in studies (4), in order to explore their impact in vivo, the genotoxicity of PhIP was investigated in
the newly established transgenic model. Line tgl was used because the strong expression was likely to facilitate the identification of trans-
gene-related effects. After oral application of PhIP, a remarkable change in the organotropism of resulting DNA adducts was observed in the transgenic animals. The liver exhibited the highest level among the tissues studied in the transgenic mice, but—in accordance with earlier studies in rodents (35,36)—represented the organ with the lowest DNA adduct level in the wild-type. It is probable that PhIP is primarily converted to N2-OH-PhIP in liver due to its high CYP1A2 level (37). Our results suggest that hSULT1A1/1A2 are very efficient in converting hepatic N2-OH-PhIP into a genotoxicant—in contrast to murine hepatic phase-2 enzymes. In particular, mSULT1A1, which is highly expressed in liver, did not activate N2-OH-PhIP in vitro (15). Therefore, other phase-2 enzymes were required for the activation in
wild-type mice, for example mSULT1D1 (15), a form that is expressed at much higher levels in many extrahepatic tissues than in liver (38). Thus, the tissue distribution of PhIP–DNA adducts in the wild-type may be explained by a relatively low hepatic activation of N2-OH-PhIP resulting in export of unmetabolized N2-OH-PhIP to be activated by endogenous SULTs and/or NATs in extrahepatic tissues. The level of circulating N2-OH-PhIP must have been lower in the transgenics because some N2-OH-PhIP was immediately trapped by sulfo-conjugation in liver. Nevertheless, the DNA adduct levels were higher in extrahepatic tissues of transgenics than in the wild-type, indicating more efficient local activation by hSULT1A1/1A2.

The strongest increase in adduct level in extrahepatic organs was observed in the lungs, the tissue passed first by blood coming from liver. Possibly, some N2-SO2-PhIP generated in the liver reached the lungs. Likewise, N2-OH-PhIP released from liver may be preferentially activated in lungs of transgenic animals, as expression of hSULT 1A1/1A2 is high in this tissue. Alternatively, the constitutive CYP1A1 expression in murine lung (39) may be important. This enzyme shows substantial N-hydroxylation activity with PhIP (39,40). Since human lung is not only exposed to orally ingested PhIP but also to PhIP inhaled, for example with cigarette smoke or cooking fumes (41,42), and pulmonary CYP1A1 expression is strongly inducible (39), lung might be an explicit target tissue of PhIP-related genotoxicity in humans.

SULTs are not the only host factor involved in the activation and/or inactivation of PhIP. In particular, marked interspecies differences were also reported for the first step in the activation of PhIP. In humans, the predominant phase-1 reaction is the formation of N2-OH-PhIP. In rodents, N2-OH-PhIP is formed to a lesser extent due to extensive ring hydroxylation generating 4′-OH-PhIP (43). The influence of phase-1 metabolism on PhIP–DNA adduct formation was addressed in Cyp1a1 (39) and Cypla2 knock-out mice (36) as well as in mice humanized for Cyp1a2 (44). However, all these genetic manipulations led to relatively moderate (~2-fold) changes in levels of PhIP-induced DNA adducts. Moreover, all changes were similar in the various tissues studied. Thus, the organotropism of PhIP was not affected in contrast to our study.

The liver of conventional mouse and rat strains is a major target tissue of carcinogenicity for many heterocyclic aromatic amines (with the notable exception of PhIP) (41). The tissue distribution of the DNA adducts formed has been studied for some hepatocarcinogenic compounds, for the notable exception of PhIP) (41). The tissue distribution of the DNA adducts formed has been studied for some hepatocarcinogenic compounds, for example 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) (45), and 2-amino-3-methyl-9H-pyrido[2,3-b]indole (MeAρC) (46) in the rat and for 2-amino-9H-pyrido[2,3-b]indole (AρC) in the mouse (47). In all these studies, liver was the tissue with clearly the highest level of DNA adducts. In contrast, PhIP only forms very low levels of adducts in the liver of conventional mice ([36] and this study) and rats (35). Adduct levels were much higher in mouse small intestine ([36] and this study) and rat colon (35), which represent major target tissues of its carcinogenicity. Based on these observations, we hypothesize that PhIP will induce liver tumors in transgenic mice for hSULT1A1/1A2, which of course has to be verified experimentally. Other tissues may also be responsive. However, every tissue with high adduct levels may not develop tumors. For instance, high levels of DNA adducts, but no significant tumor induction have been observed in the pancreas of PhIP-treated rats (35).

With the limitation, that DNA damage is only one factor in carci-
nogenesis, we could show that the expression of human SULTs affects the extent and the organotropism of PhIP-induced genotoxicity. The transgenic lines therefore represent a valuable model for further in vivo investigations of the hSULT1A1/1A2 influence on the geno-
toxicity and carcinogenicity of various food-borne or environ-
mental xenobiotics.

Supplementary material

Supplementary Tables 1 and 2 and Figures 1–3 can be found at http://
carcin.oxfordjournals.org/
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References