IMPORTANCE OF UDP-GLUCURONOSYLTRANSFERASE 1A10 (UGT1A10) IN THE DETOXIFICATION OF POLYCYCLIC AROMATIC HYDROCARBONS: DECREASED GLUCURONIDATIVE ACTIVITY OF THE UGT1A10<sup>139LYS</sup> ISOFORM

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ABSTRACT:

UDP-glucuronosyltransferase 1A10 (UGT1A10) is an extrahepatic enzyme expressed in aerodigestive tract tissues that exhibits significantly glucuronidation activity against the important procarcinogenic benzo(a)pyrene (BaP) metabolite, BaP-trans-7,8-dihydrodiol (BPD), and the UGT1A10 codon 139 (Glu<sup>−</sup>Lys) polymorphism was previously implicated in risk for orolaryngeal cancer by Elahi et al. in their 2003 study. To better assess the potential role of UGT1A10 in risk for tobacco-related cancers, the glucuronidation activity of UGT1A10 was compared with that of other known UGT enzymes against selected polycyclic aromatic hydrocarbons, and the effects of the codon 139 polymorphism on UGT1A10 function were examined in vitro. UGT1A10 exhibited considerably more glucuronidation activity as determined by \( V_{\text{max}}/K_m \) against 3-hydroxy (OH)-BaP, 7-OH-BaP, 9-OH-BaP, and 1-OH-pyrene than any other UGT1A family member. Although a kinetic comparison using \( V_{\text{max}} \) could not be performed against family 2B UGTs, UGT1A10 exhibited a 1.7- to 254-fold lower \( K_m \) than active family 2B UGTs against 3-OH-BaP, 7-OH-BaP, and 1-OH-pyrene. A significantly (\( p < 0.01 \)) higher \( V_{\text{max}}/K_m \) was observed for homogenates from wild-type UGT1A10<sup>139Glu</sup>-overexpressing cells against all four BaP metabolites tested (3-OH-BaP, 7-OH-BaP, 9-OH-BaP, and BPD). A similarly significant (\( p < 0.05 \)) increase in \( V_{\text{max}}/K_m \) was observed for homogenates from wild-type UGT1A10<sup>139Glu</sup>-overexpressing cells against 1-OH-pyrene. Significant differences in \( K_m \) were observed for homogenates from wild-type UGT1A10<sup>139Glu</sup>-overexpressing cells against 1-OH-pyrene (\( p < 0.05 \)) and 3-OH-BaP (\( p < 0.01 \)). Reverse transcription-polymerase chain reaction of total lung RNA showed low levels of UGT1A10 expression in human lung tissue. Together, these studies implicate UGT1A10 as an important detoxifier of polycyclic aromatic hydrocarbons in humans and that the UGT1A10 codon 139 polymorphism may be an important determinant in risk for tobacco-related cancers.

The UDP-glucuronosyltransferase (UGT) superfamily of enzymes catalyzes the glucuronidation of a variety of endogenous compounds such as bilirubin and steroid hormones, as well as xenobiotics such as drugs and environmental carcinogens (Tephly and Burchell, 1990; Owens and Ritter, 1995; Gueraud and Paris, 1998; Ren et al., 2000). Based on structural and sequence homology, UGTs are classified into several families and subfamilies (Jin et al., 1993). UGT family 2B members are derived from independent genes, whereas the entire UGT1A family is derived from a single gene locus in chromosome 2. This locus codes for nine functional proteins that differ only in their amino terminus as a result of alternate splicing of independent exons 1 regions to a shared carboxy terminus encoded by exons 2 through 5 (Beaulieu et al., 1997). Several family 1A UGTs have been implicated in the detoxification of tobacco carcinogen metabolites including the tobacco-specific nitrosamine, NNK (Ren et al., 2000; Wiener et al., 2004b), and polycyclic aromatic hydrocarbons (PAHs) like benzo(a)pyrene (BaP) (Ciotti et al., 1997; Levesque et al., 1997; Beaulieu et al., 1998; Bélanger et al., 1998; Carrier et al., 2000). Although most family 1A UGTs are expressed in the liver (Ciotti et al., 1997; Burchell and Hume, 1999; Levesque et al., 1999; Guillemette et al., 2000), several UGTs are extrahepatic (Tukey and Strassburg, 2000) and are expressed in several target tissues for tobacco-induced cancers, including tissues in the aerodigestive tract (Zheng et al., 2002). Polymorphisms have been previously identified for many of the UGT genes, and several recent studies have examined their potential role in tobacco carcinogenesis and in risk for tobacco-induced cancers. In studies examining UGT family 1A variants, the “TATA” box polymorphism in the promoter region of UGT1A1, commonly associated with Gilbert’s syndrome, is associated with reduced function in the UGT1A1 transcriptional promoter (Burchell and Hume, 1999) and is associated with decreased formation of the glucuronide conjugate of

**ABBREVIATIONS:** UGT, UDP-glucuronosyltransferase; NNK, 4-(methylamino)-1-(3-pyridyl)-1-butanone; PAH, polycyclic aromatic hydrocarbon; BaP, benzo(a)pyrene; BPD, BaP-trans-7,8-dihydrodiol; NNAL, 4-(methylamino)-1-(3-pyridyl)-1-butanol; 3-OH-BaP, 3-hydroxybenzo(a)pyrene; 7-OH-BaP, 7-hydroxybenzo(a)pyrene; 9-OH-BaP, 9-hydroxybenzo(a)pyrene; UDPGA, UDP-glucuronic acid; 1-OH-pyrene, 1-hydroxypyrene; HEK, human embryonic kidney; PCR, polymerase chain reaction; RT, reverse transcription.
the important procarcinogenic BaP metabolite, BaP-trans-7,8-dihydrodiol (BPD), in liver microsomes (Fang and Lazarus, 2004). UGT1A7-specific genetic variants are associated with reduced UGT1A7 metabolic function against BaP phenols (Guillemette et al., 2000) and are strongly linked to increased risk for orolaryngeal cancer (Zheng et al., 2001; Ockenga et al., 2003), and lung (Araki et al., 2005) cancer. Recent studies have shown an association between liver microsomal O-glucuronide conjugate formation activity against NNAL, the major metabolite of NNK, and a Pro->Thr polymorphism at codon 24 of the UGT1A4 gene (Wiener et al., 2004a). Among the family of 2B polymorphic variants, recent studies have shown an association between both the UGT2B7 codon 268 polymorphism and the UGT2B17 gene deletion polymorphism and the O-glucuronidation of NNAL in liver microsomes (Wiener et al., 2004a; Lazarus et al., 2005).

UGT1A10 is an extrahepatic enzyme expressed in aerodigestive tract tissues (Zheng et al., 2002) that exhibits significant glucuronidation activity against BPD (Fang et al., 2002). Previous studies have shown that polymorphism in codon 139 of the UGT1A10 gene, resulting in a nonconservative Glu->Lys amino acid change, was linked to altered risk for orolaryngeal cancer (Elahi et al., 2003). No such association was observed for the UGT1A10 codon 244 polymorphism, which results in a conservative amino acid change of Leu->Ile. To better assess the potential role of UGT1A10 in risk for tobacco-related cancers, the glucuronidation activity of UGT1A10 was compared with that of other known UGT enzymes against selected PAHs, UGT1A10 expression was examined in lung tissue, and the effects of the codon 139 polymorphism on UGT1A10 function were examined in vitro. Results are presented showing that UGT1A10 appears to be the most highly active UGT against a number of tobacco carcino- gen metabolites, that UGT1A10 is expressed in lung, and that the UGT1A10139Glu-encoded variant exhibits reduced enzyme activity against all the substrates tested compared with the wild-type UGT1A10139Glu-encoded isoform in vitro.

Materials and Methods

Chemicals and Materials. 3-Hydroxy (OH)-BaP, 7-OH-BaP, 9-OH-BaP, and BPD were obtained from the National Cancer Institute Chemical Carcinogen Repository (Midwest Research Institute, Kansas City, MO). UDP-glucuronic acid (UDPGA), N,N,N,N-tetraisopropylphosphatidyl choline palmital C16:0, 1-hydroxypyrene (1-OH-pyrene), 1-naphthol, 4-nitrophenol, and 4-methylumbelliferone were purchased from Sigma (St. Louis, MO). 13C-UDPGA (specific activity: 300 mCi/mmol) was obtained from PerkinElmer (Wellesley, MA). Dulbecco’s modified Eagle’s medium was obtained from Mediatech (Herndon, VA), and both fetal bovine serum and geneticin (G418) were purchased from Invitrogen (Carlsbad, CA). Taq DNA polymerase (HotMaster) was purchased from PerkinElmer Biosystems (Foster City, CA); Moloney murine leukemia virus reverse transcriptase and the pdcDNA3.1/V5-His-TOPO mammalian expression vector were obtained from Invitrogen (Carlsbad, CA); the human UGT1A10 Western blot kit that includes the anti-UGT1A10 polyclonal antibody was purchased from BD Gentest (Woburn, MA), and the anti-β-actin monoclonal antibody (1:5000 dilution) was provided by Sigma.

The human embryonic kidney (HEK) 293 cell line was purchased from The American Type Culture Collection (Manassas, VA), and the human oral squamous cell carcinoma SKM1483 cell line was obtained by Peter Sacks (New York University, New York, NY). Cell lines stably overexpressing UGT1A4, UGT1A6, UGT1A8, UGT2B4, and UGT2B7 were described previously (Ren et al., 2000; Wiener et al., 2004b). Baculosomes (BD Gentest) were used for screening UGT1A1, UGT1A3, UGT1A7, UGT1A9, UGT2B15, and UGT2B17 glucuronidation activities.

UGT1A10 Cloning and Reverse Transcription-Polymerase Chain Reaction of Lung Tissue. The amplification of UGT1A10 cDNA was performed after an initial reverse transcriptase reaction using 3 μg of total RNA from the SKM1483 cell line (shown previously to express UGT1A10; P. Lazarus, unpublished data), 2.5 μM oligo(dT) primer, and 200 units of reverse transcriptase in a 50-μl incubation at 42°C. Polymerase chain reaction (PCR) amplification was subsequently performed using 2 μl of the reverse transcriptase reaction in a 50-μl reaction volume containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl2, 0.2 mM concentration each of deoxynucleotide triphosphates, 20 pmol of both sense (UGT1A10s1, 5’-TCTGCCCTACCTG-TATCATAGCAG-3’), corresponding to nucleotides +6 to +25 relative to the UGT1A10 translation stop codon) primers, and 2.5 units of Pfu DNA polymerase. Incubations were performed in a GeneAmp 9700 thermocycler (Applied Biosystems, Foster City, CA) as follows: 1 cycle of 94°C for 2 min, 41 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 2 min, followed by a final cycle of 7 min at 72°C. The PCR product (1679 bp) was purified after electrophoresis in 1.5% agarose using the QIAEX II gel extraction kit (QIAGEN, Valencia, CA) and subsequently subcloned into the pdcDNA3.1/V5-His-TOPO mammalian expression vector using standard methodologies. Confirmation of insert orientation was performed by restriction enzyme digestion, and the UGT1A10 sequence was confirmed by dyeoxy sequencing of the entire PCR-amplified UGT1A10 cDNA product (performed at the Molecular Biology Core Facility at Penn State University College of Medicine) using two vector primers (T7 and BGH; Integrated DNA Technologies, Coralville, IA) and one internal sense primer (UGT1A10s2S; 5’- CCTCTTCTCTATGCTCCCAATGTA-3’), corresponding to nucleotides +556 to +578 relative to the UGT1A10 translation start site in the UGT1A10 cDNA by comparison with the UGT1A10 cdNA sequence described in GenBank accession no. BC020971.

Reverse transcription (RT)-PCR from normal human lung tissue was performed as described above except that total RNA from histologically normal human lung tissue specimens, obtained from the Tissue Procurement Facility at the H. Lee Moffitt Cancer Center (Tampa, FL), was used as a template. The primers used to amplify exon 1 of UGT1A10 were described previously (Zheng et al., 2002). All the protocols involving the analysis of tissue specimens were approved by the Institutional Review Board at Penn State College of Medicine and in accordance with assurances filed with and approved by the U.S. Department of Health and Human Services.

Site-Directed Mutagenesis, Generation of Cell Lines, and Cell Homogenate Preparation. The UGT1A10139Glu variant was generated by PCR amplification of the pdcDNA3.1/V5-His-TOPO vector containing the wild-type UGT1A10 sequence using site-directed mutagenesis primers specific for the polymorphic site. The primers used to generate this variant were UGT1A10-m139F (5’-GTAGAATACTTAAAGAAGGTCTTTGTTGACGTTGTCG-3’) and UGT1A10-m139R (5’-CCAGAACAATCGTCATCAAAGAATCTTCTTTAGTATCTAC-3’), corresponding to bases +400 to +442 relative to the UGT1A10 translation start site, with the polymorphic base in bold for both primers. PCR was performed using 10 units of Pfu polymerase, 1X Pfu buffer, 2 mM dNTPs in 50 μl of template (1 mM MgSO4, 300 μM deoxynucleoside-5’-triphosphate, and 0.6 to 2.4 μM concentrations of each primer. The products were amplified in a Bio-Rad (Hercules, CA) MyCycler with an initial denaturation of 95°C for 2 min, followed by 25 cycles of 95°C for 30 s, 59°C to 61°C for 30 s, and 68°C for 18 min. After amplification, 20 units of the DpnII restriction enzyme were added to each reaction and incubated at 15 h at 37°C to specifically digest the wild-type template DNA. The nondigested, PCR-amplified plasmid (which has incorporated the polymorphism) was then transformed into competent DH5α Escherichia coli; individual colonies were isolated; and subsequent plasmid DNA minipreps were screened for the codon 139 variant by digestion with the Earl restriction enzyme, which specifically recognizes the UGT1A10139Glu but not the UGT1A10139Glu variant. UGT1A10 sequences were confirmed by dyeoxy DNA sequencing analysis using the same primers used to confirm the cloning of wild-type UGT1A10 as described above.

HEK293 cell lines overexpressing wild-type or variant UGT1A10 were generated by stable transfection using the LipofectAMINE reagent (Invitrogen) procedure according to the manufacturer’s protocol. Briefly, pdcDNA3.1/V5-His-TOPO/UGT1A10 constructs were transfected into HEK293 cells grown in 5% CO2 to 80% confluence in Dulbecco’s modified Eagle’s medium supplemented with 4.5 mM glucose, 10 mM HEPES, 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin. At 24 h post-transfection,
cells were passaged and subsequently grown in genetin (700 μg/ml medium) for the selection of genetin-resistant cells, with selection medium changed every 3 to 4 days. Cell homogenates were prepared by resuspending pelleted cells in Tris-buffered saline (25 mM Tris base, 138 mM NaCl, 2.7 mM KCl, pH 7.4) and subjecting them to three rounds of freeze-thaw before gentle homogenization. Cell homogenates (5–30 mg/ml homogenate protein) were stored at −70°C in 100-μl aliquots. Total cell homogenate protein concentrations were determined using the BCA assay from Pierce Biotechnology (Rockford, IL) after protein extraction using standard protocols.

**Western Blot Analysis.** Levels of UGT1A protein in UGT-overexpressing cell lines were measured by Western blot analysis using the anti-UGT1A antibody (1:5000 dilution as per the manufacturer’s instructions), whereas β-actin protein levels were assayed using a 1:5000 dilution of the monoclonal anti-β-actin antibody. UGT1A protein was detected by chemiluminescence using the SuperSignal West Dura Extended Duration Substrate (Pierce Biotechnology). Secondary antibodies supplied with the Dura enhanced chemiluminescence kit (anti-rabbit and anti-mouse) were used at 1:3000. UGT1A1 protein levels were quantified against a known amount of human UGT1A1 (Bio-Rad). Quantification was made relative to the levels of β-actin observed in each lane (also quantified by densitometric analysis of Western blots as described previously (Ren et al., 2000; Fang et al., 2002; Wiener et al., 2004b)). X-ray film bands were always below densitometer saturation in each lane (also quantified by densitometric analysis of X-ray film exposures (5-s to 2-min exposures) of Western blots using a GS-800 densitometer with Quantity One software (Bio-Rad). Quantification was made relative to the levels of β-actin observed in each lane (also quantified by densitometric analysis of Western blots as described previously (Ren et al., 2000; Fang et al., 2002; Wiener et al., 2004b)).
V_{\text{max}}/K_m against 1-OH-pyrene in addition to UGT1A10 were UGT1A6 and UGT1A8. Although a comparison using \( V_{\text{max}} \) could not be performed against family 2B UGT, UGT1A10 exhibited a 1.7- to 254-fold lower apparent \( K_m \) than active family 2B UGTs against 3-OH-BaP, 7-OH-BaP, and 1-OH-pyrene. Overall, the affinity of individual UGT enzymes as determined by apparent \( K_m \) analysis was 1A10 > 1A1 > 1A9 > 1A7 > 1A8 > 2B17 > 1A3 > 2B15 > 2B7 for 3-OH-BaP, 1A10 > 1A3 > 1A9 > 1A7 > 2B7 > 1A8 > 1A1 > 2B15 > 2B7 for 7-OH-BaP, 1A7 > 1A10 > 2B7 > 2B15 > 1A9 > 1A3 > 1A1 > 2B17 > 1A8 for 9-OH-BaP, and 1A10 > 1A9 > 1A7 > 2B17 > 1A1 > 1A6 > 2B15 > 1A8 > 1A3 > 2B7 for 1-OH-pyrene.

Expression of UGT1A10 in Human Lung. Previous studies have shown that UGT1A10 and UGT1A7 are the only UGTs expressed in all the aerodigestive tract tissues examined, including floor of mouth, larynx, tonsil, esophagus, and tongue (Zheng et al., 2002). In the same studies, UGT1A10 was not detected in lung tissue by multiplex RT-PCR with primers for 3-OH-BaP, 7-OH-BaP, and 1-OH-pyrene. Overall, the affinity of family 1A UGTs, varying amounts of total protein were loaded as indicated. The relative densitometric values are also given for the representative blot shown setting the UGT1A1 standard to 1.0. * Fold-difference values are the average of three independent Western blot experiments. The relative ratios of UGT1A10/β-actin levels are shown under the corresponding lanes for each of the UGT1A10-overexpressing cell lines, with the UGT1A10/β-actin ratio for the UGT1A10\(^{139\text{Glu}}\)-overexpressing cell protein lysate designated as 1.0 as reference. One hundred nanograms of UGT1A10 protein standard (from BD Gentest) was loaded as a gel-loading reference.

Analysis of UGT1A10\(^{139\text{Glu}}\) Function Using UGT1A10-Overexpressing Cell Lines. To determine whether the Glu>Lys amino acid change at codon 139 of the UGT1A10 gene affects UGT1A10 enzyme activity, stable HEK293 cell lines overexpressing either the UGT1A10\(^{139\text{Glu}}\) or UGT1A10\(^{139\text{Lys}}\) isoforms were created. The overexpressed UGT1A10 wild-type and polymorphic cDNA were PCR-amplified and fully sequenced as described under Materials and Methods. The UGT1A10 cDNA sequence of both cell lines matched with 100% identity to the UGT1A10 cDNA sequence described in GenBank (accession no. BC030974) with the only difference between the cell lines being the polymorphic G>A transition at nucleotide
UGT1A10139Lys-overexpressing HEK293 cell lines (Fig. 1C). The glucuronidation activity was clearly discernable for both the UGT1A10139Glu and UGT1A10139Lys-overexpressing cell homogenate. The differences in activity were more evident with treatment with 1-OH-pyrene (Table 2). Between 2.1- and 2.9-fold less activity was observed for UGT1A10139Glu-overexpressing cell homogenates as determined by Western blot analysis. Glucuronidation assays were performed using 0.5 mg of UGT1A10-overexpressing cell homogenate for a 1-h incubation at 37°C. Aryl glucuronidase concentrations were 1 mM.

**Discussion**

UGT1A10 is an extrahepatic enzyme that is expressed in tissues of the digestive tract (Strassburg et al., 1999) and aerodigestive tract (Zheng et al., 2002). UGT1A10 has been implicated in the glucuronidation of several important carcinogens that probably play an important role in cancer initiation at these sites, including PAH-like BaP-7,8-dihydrodiol, precursor to the ultimate carcinogenic metabolite of BaP, BaP-7,8-dihydrodiol-9,10-epoxide (Fang et al., 2002), and 2-hydroxyamino-1-methyl-6-phenylimidazo[4,5-b]pyridine (N-hydroxy-PhIP), the reactive intermediate of PhIP, a heterocyclic amine found in abundance in charbroiled meats (Malfatti and Felton, 2004). Previous reports identified two missense polymorphisms for UGT1A10, one at codon 390 that results in a nonconservative change in amino acids Glu to Lys (Elahi et al., 2003). Both polymorphisms were somewhat prevalent (0.04–0.05) in African Americans but much less prevalent in other racial groups, including Caucasians (<0.01 for both groups). In a small hospital-based case/control study of 230 African American subjects, only the codon 139 polymorphism was associated with orolaryngeal cancer risk, with subjects with the UGT1A10139Lys variant exhibiting a significantly decreased risk for orolaryngeal cancer (Elahi et al., 2003).

The purpose of the present study was to further evaluate the role of UGT1A10 as a potentially important enzyme in the metabolism of PAH and to examine the effects of the UGT1A10 codon 139 polymorphism on UGT1A10 activity against the same PAH in vitro. Results presented here clearly show that of all the UGTs examined, UGT1A10 exhibits the highest glucuronidation activity against the various PAHs tested. These results are consistent with the high relative activity previously observed for UGT1A10 against both the (+)
and (−) isomers of BPD (Fang et al., 2002). This suggests that UGT1A10 could be playing a major role in the detoxification of PAH in important target sites, including tissues within the digestive and aerodigestive tracts, where exposure to PAH via charbroiled meats or tobacco smoke may be important in cancer induction.

Results from this study also showed detectable levels of expression of UGT1A10 in lung tissue, indicating that UGT1A10 may also be playing a role in the detoxification of tobacco smoke PAH within the lung. The fact that UGT1A10 was not observed to be expressed in lung tissue in previous studies is likely because of differences in assay sensitivity because previous studies were performed using multiplex RT-PCR using β-actin as an internal control for amplification (Zheng et al., 2002). Although excellent for quality control, the use of multiple primers for the amplification of multiple genes in single PCR reactions can affect overall amplification sensitivity and can interfere with the amplification of low-copy number mRNA (Markoulatos et al., 2002), a possibility for UGT1A10 in lung. Alternatively, it is possible that UGT1A10 may be localized to different regions within the lung, and that the lung specimens examined in the two studies were from different lung sites, or that UGT1A10 may have been differentially induced in the lungs from the different individuals from whom the specimens were obtained. Future studies will be necessary to evaluate these possibilities.

Results from the present study show that the UGT1A10139Lys polymorphic variant exhibits significantly decreased glucuronidation activity against several PAHs, including metabolites of BaP and 1-OH-pyrene in vitro. The decreased activity observed for UGT1A10139Glu cell homogenates against these PAHs was reflected by significantly decreased \( V_{\text{max}} \) and, in some cases, significantly higher apparent \( K_m \), compared with UGT1A10139Glu cell homogenates. Similarly significant decreases in glucuronidation activity were observed for UGT1A10139Lys cell homogenates against all the noncarcinogenic aglycones examined in this study. The fact that there were differences in the apparent \( K_m \) for the wild-type UGT1A10 against BPD in the present study versus previous studies (Fang et al., 2002) is likely because UGT1A10-overexpressing baculosomes were used in previous studies, whereas a UGT1A10-overexpressing cell line was used in the present analysis. Recent studies have suggested that UGT1A10 activity is phosphorylation-dependent, potentially involving kinases that may not be present or as active in insect cells versus human cells (Basu et al., 2004).

Together, these results suggest that the nonconservative amino change of Glu to Lys at codon 139 renders UGT1A10 less active. Interestingly, this decrease in glucuronidation activity for the UGT1A10139Lys variant is not consistent with the protective effect observed for this allele in orolaryngeal cancer case/control studies (Elahi et al., 2003). One possibility that could explain this is that the two variants may have different optimal assay conditions in vitro. Another possibility is that the previous case/control study was small (115 individually matched African American case/control pairs) and that the association observed was an aberration. However, credence to the results of this study was provided by the fact that an association with orolaryngeal cancer risk was not observed with the more conservative codon 244 missense UGT1A10 polymorphism examined in the same population. The most likely possibility that could explain the differences observed between these molecular epidemiologic studies and the functional studies outlined in this report is that the UGT1A10139Lys allelic variant may be linked by haplotype to one or more other genetic variations within the UGT1A loci that not only compensate for the detrimental effect on UGT1A10 function by the presence of the codon 139 Lys residue but also serve to provide an overall protective effect against orolaryngeal cancer risk. A haplotype analysis of potential variants within the UGT1A10 coding region was performed in previous studies, where the UGT1A10-specific exon 1 was screened for polymorphisms in a total of 102 subjects (53 Caucasians and 49 African Americans) (Elahi et al., 2003). The allelic variant encoding the UGT1A10139Lys isoform (termed UGT1A10*2) was not observed to be linked to any other UGT1A10 exon 1 polymorphisms in these studies. Alternatively, linkage could be with variants within the UGT1A10 promoter region, sequences that were not examined in these previous studies, or in linkage with other genetic variations within the UGT family 1A loci that somehow affect UGT1A10 expression. Another possibility is that the UGT1A10 codon 139 polymorphism may be linked to functionally relevant polymorphism(s) in another family 1A UGT that is important in orolaryngeal cancer risk. Previous studies have shown that function-altering polymorphisms in the UGT1A7 gene are strongly associated with tobacco smoking-related orolaryngeal cancer risk in both Caucasian and African Americans (Zheng et al., 2001). Like UGT1A10, UGT1A7 was also shown to be the only other UGTs to be well expressed in all the aerodigestive tract tissues examined (Zheng et al., 2002) and, as described in this and previous reports (Fang et al., 2002; Guillemette et al., 2000), UGT1A7 exhibits glucuronidating activity against various PAHs including BPD. It is not yet known whether a genetic linkage exists between the UGT1A10 codon 139 polymorphism and polymorphisms in other regions within the UGT family 1A loci including UGT1A17. Studies attempting to identify UGT1A10 codon 139-inclusive haplotypes within the UGT family 1A locus by large-scale sequencing analysis of individuals with a UGT1A10*2 allele are currently in progress.

In summary, UGT1A10 is the most active UGT in the glucuronidation of various PAHs and is expressed in various target tissues where PAH exposure may be important in cancer induction, including tissues within the digestive and aerodigestive tracts and potentially within the

### TABLE 3

Kinetic analysis of glucuronide formation for BaP metabolites for UGT1A10139Glu- and UGT1A10139Lys-overexpressing cell homogenates

<table>
<thead>
<tr>
<th>Substrate</th>
<th>( K_m ) (μM)</th>
<th>( V_{\text{max}} ) (nmol·min(^{-1}·mg) (^{-1}))</th>
<th>( V_{\text{max}}/K_m ) (μmol·min(^{-1}·mg) (^{-1}))</th>
<th>( V_{\text{max}}/K_m ) (μmol·min(^{-1}·mg) (^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>BaP</td>
<td>46.8 ± 4.2</td>
<td>7.7 ± 0.4</td>
<td>165 ± 6.5</td>
<td>15.4 ± 1.6(^{a})</td>
</tr>
<tr>
<td>3-OH-BaP</td>
<td>9.7 ± 1.2</td>
<td>24.8 ± 1.9</td>
<td>2557 ± 124</td>
<td>17.7 ± 1.2(^{a})</td>
</tr>
<tr>
<td>7-OH-BaP</td>
<td>9.8 ± 0.5</td>
<td>25.8 ± 0.7</td>
<td>2633 ± 81</td>
<td>13.2 ± 0.8(^{a})</td>
</tr>
<tr>
<td>9-OH-BaP</td>
<td>38.2 ± 6.2</td>
<td>69.4 ± 9.2</td>
<td>1817 ± 59</td>
<td>44.8 ± 2.4</td>
</tr>
<tr>
<td>1-OH-pyrene</td>
<td>11.3 ± 3.3</td>
<td>90.0 ± 2.4</td>
<td>7965 ± 2100</td>
<td>50.4 ± 3.1(^{b})</td>
</tr>
</tbody>
</table>

\(^{a}\) Significant \((\ p < 0.01 \text{ or } p < 0.05 \text{ })\) increase in kinetic parameter observed for homogenates from UGT1A10139Glu- versus UGT1A10139Lys-overexpressing cells.
lung. Further investigations will be required to fully elucidate the mechanism underlying the observed protective phenotype in orolaryngeal cancer risk associated with the functionally less-active UGT1A10*39L-lys polymorphic variant.

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