Published in final edited form as:

Steroids. 2008 July ; 73(6): 611–620.

IDENTIFICATION OF UDP-GLUCURONOSYLTRANSFERASE 1A10 IN NON-MALIGNANT AND MALIGNANT HUMAN BREAST TISSUES

Athena Starlard-Davenport¹, Beverly Lyn-Cook², and Anna Radomska-Pandya^{1†}

¹Department of Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences, Little Rock, AR 72205, USA

²Division of Personalized Nutrition and Medicine, National Center for Toxicological Research, HFT-100, Jefferson, AR 72079, USA

Abstract

UGT1A10 was recently identified as the major isoform that conjugates estrogens. In this study, real-time PCR revealed high levels of UGT1A10 and UGT2B7 in human breast tissues. The expression of UGT1A10 in breast was a novel finding. UGT1A10 and UGT2B7 mRNAs were differentially expressed among normal and malignant specimens. Their overall expression was significantly decreased in breast carcinomas as compared to normal breast specimens (UGT1A10: 68 ± 26 vs. 252 ± 86 , respectively; $p < 0.05$) and (UGT2B7: 1.4 ± 0.7 vs. 12 ± 4 , respectively; $p < 0.05$). Interestingly, in African American women, UGT1A10 expression was significantly decreased in breast carcinomas in comparison to normals (57 ± 35 vs. 397 ± 152 , respectively; $p < 0.05$). Among Caucasian women, UGT2B7 was significantly decreased in breast carcinomas in comparison to normals (1.1 ± 0.5 vs. 13.5 ± 6 , respectively; $p < 0.05$). Glucuronidation of 4-OHE₁ was significantly reduced in breast carcinomas compared to normals (30 ± 15 vs. 106 ± 31 , respectively; $p < 0.05$). Differential down-regulation of UGT1A10 and UGT2B7 mRNA, protein, and activity in breast carcinomas compared to the adjacent normal breast specimens from the same donor were also found. These data illustrate the novel finding of UGT1A10 in human breast and confirm the expression of UGT2B7. Significant individual variation and down-regulation of expression in breast carcinomas of both isoforms was also demonstrated. These findings provide evidence that decreased UGT expression and activity could result in the promotion of carcinogenesis.

Keywords

UGTs; UGT1A10; breast cancer; African Americans; Caucasians

INTRODUCTION

Glucuronidation, catalyzed by UGTs, is an important process for the detoxification of many important endogenous compounds, various drugs, and xenobiotics. Glucuronidation facilitates the elimination of inactive hydrophilic glucuronides from the body via urine or bile [1,2]. In

†Corresponding author: Anna Radomska-Pandya, Ph.D., Dept. of Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences, 4301 W. Markham, Slot 516, Little Rock, AR 72205, Tel: (501)-686-5414, Fax: (501)-603-1146, Email: radominskaanna@uams.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

general, glucuronidation is thought to serve as an inactivating or protective function by terminating or attenuating the activity of many endogenous, dietary and clinically administered drugs as well as environmental chemicals that have been shown to result in toxicity or carcinogenesis [2]. However, glucuronidation also can result in the generation of bioactive or even toxic compounds, including those of estrogens, morphine, retinoids, bile acids, and heterocyclic aromatic amines [3–7].

UGTs have been classified into two families, UGT1 and UGT2, based on the similarity of their primary amino acid sequence [8]. In humans, the UGT1 gene locus encodes RNA transcripts encoded by a single gene locus on chromosome 2-q37 [9,10]. Most of the UGT1A genes are expressed in hepatic tissues; however, UGT1A7, UGT1A8, and UGT1A10 are expressed in extrahepatic tissues, such as colon, intestine, kidney, lung, esophagus, prostate, and breast [11–13]. In contrast, individual UGT2 genes, clustered on chromosome 4q-13, each comprise six exons that, with the exception of UGT2A1 and 2A2, are not shared between the family members. UGT2B isoforms are widely distributed in human tissues such as kidney, adipose tissue, placenta, skin, lung, pancreas, prostate, and breast in addition to the liver [10]. UGTs from both families were of particular interest to us due to their direct involvement in the biotransformation of estradiol (E_2), and the 4-hydroxylated catechol-estrogens (4-OH-CEs), which are genotoxic [14].

Estrogens have been implicated in the development of carcinogenesis in estrogen target tissues. For instance, several studies have demonstrated that metabolism of estrogens in estrogen target tissues is responsible for estrogen-induced tumor development and cellular transformation [15,16]. This process can occur when oxidation of the native estrogens, estrone, E_1 , or E_2 , by cytochrome P450 enzymes generates two classes of catecholestrogens, the 2- and 4-hydroxylated CE. Oxidation of native estrogens to the 2-hydroxylated CE produces a stable, non-carcinogenic metabolite. On the other hand, generation of 4-hydroxylated CE, particularly 4-hydroxylated estrone (4-OHE₁) is associated with the development of estrogen-sensitive cancers via metabolic redox cycling [17,18]. One possible mechanism for the genotoxicity of the catechol estrogens is the generation of electrophilic metabolites that react with DNA to form depurinating DNA adducts, which ultimately leads to cancer [19].

Information on the involvement of UGTs in the detoxification of estrogens is limited; however, recent findings suggest that glucuronidation by UGTs could directly promote the removal of native estrogens and their hydroxylated metabolites from the body [14,20–22]. Recently, our laboratory has identified seven human recombinant UGTs, UGT1A1, 1A3, 1A4, 1A6, 1A7, 1A8, 1A9, 1A10, and 2B7, as being involved in the biotransformation of native estrogens and their oxidative metabolites. Of the UGTs identified, UGT1A10 has been established as the major UGT isoform that conjugates all the studied estrogens and UGT2B7 conjugated the genotoxic 4-OHE₁ with relatively high activity [20]. To date, UGT1A1, 1A3, 1A4, 1A8, 1A9, 2B4, 2B7, 2B15 and 2B28 have been characterized in breast tissues and/or breast cell lines [23–29]. However, to our knowledge, no studies have investigated the expression of UGT1A10 in any estrogen target tissue, including breast. In addition, there have been no studies that quantitatively demonstrate the mRNA levels of UGT1A10. Therefore, we undertook experiments to characterize the expression of UGT1A10 and UGT2B7, which has previously been identified [30], in normal and malignant human breast tissues by determining UGT1A10 and UGT2B7 mRNA, protein levels, and glucuronidation of two selected estrogens, E_2 and 4-OHE₁. These studies demonstrate for the first time the expression of UGT1A10 mRNA in human breast tissues and confirm the expression of UGT2B7 mRNA and protein. Moreover, we have found that the mean levels of UGT1A10 and UGT2B7 transcripts and glucuronidation activity toward 4-OHE₁ are significantly decreased in breast carcinomas as compared to normal breast tissues. Those studies suggest that these isoforms may be involved in the protection of

breast tissues from the carcinogenic effect of estrogens and that their down-regulation may promote carcinogenesis.

MATERIALS AND METHODS

Tissue Samples

A total of 61 breast specimens were obtained from the Cooperative Human Tissue Network Bank (CHTN) (Birmingham, AL). Samples (> 500 mg) were snap-frozen and stored at -80°C until analysis. Of the 61 breast specimens obtained, 30 patients had undergone reduction mammoplasty for macromastia (Normals) and 31 patients had surgical mastectomy for removal of the carcinoma. Invasive breast carcinomas obtained from CHTN were ductal ($n = 27$), lobular ($n = 3$), or a combination of ductal and lobular carcinomas ($n = 1$). All tumor specimens were grade 3. In addition, tumor and adjacent normal breast tissues, shown to be histologically free of cancer, of the same patient were obtained for these studies. In particular, adjacent normal tissue was excised away from the cancer lesion macroscopically, and their histological diagnosis was confirmed microscopically. Adjacent normal breast tissues were defined as histological normal breast tissues, as determined by a pathologist, isolated near the site of the breast cancer from the same donor. Non-cancerous, adjacent breast specimens obtained from the CHTN were diagnosed as either normal or malignant by a certified pathologist as stated in pathological reports. The age of patients with macromastia ranged from 18 to 76 years and that of patients with invasive breast cancers ranged from 29 to 79 years. Characteristics of donors used in these studies are detailed in Table 1. Authorization for the use of these tissues for research was obtained from the Institutional Review Board for use of Human Tissues at the University of Arkansas for Medical Sciences, Little Rock, Arkansas.

RNA Extraction

Total RNA was isolated from frozen tissues using the Qiagen RNA Isolation Kit (Valencia, CA) according to the manufacturer's instructions. RNA isolated from breast samples were dissolved in RNase-free water and quantified by spectrophotometric readings at 260 nm (A_{260}). The quality of total RNA samples was determined by the A_{260}/A_{280} ratio, and their integrity was confirmed by electrophoresis on 1 % agarose gels.

cDNA Synthesis

Total RNA (2 μg) isolated from normal and malignant breast tissues was reverse transcribed in a final volume of 20 μl containing 5 X RT-PCR buffer, 10 mM each deoxynucleotide triphosphate, 40 units/ μL of recombinant RNase inhibitor, 200 units/ μL of MMLV reverse transcriptase, and 20 μM random hexamers. Samples were incubated at 42°C for 60 min., reverse transcriptase was inactivated by heating at 70°C for min and cooling at 4°C for 1 min.

Quantitative Real-Time PCR (QRT-PCR)

PCR reactions were performed in a volume of 50 μl containing equal amounts of cDNA (50 ng) from each breast specimen, 400 nM of each primer, deionized water, and 25 μl SYBR green Master Mix (Stratagene, Cedar Creek, TX) using the following conditions: 50°C for 2 min and denaturing at 95°C for 10 min, followed by 40 cycles of 95°C for 15s and 60°C for 1min. PCRs were carried out in 96-well thin-wall PCR plates covered with optically clear sealing film (Applied Biosystems). Amplification, detection, and data analysis were performed using the ABI PRISM 7000 Sequence Detector system (Applied Biosystems). Previously published UGT primer sequences used for real-time PCR are given in Table 2 [31]. Results were expressed using the comparative threshold method, following the recommendations of the manufacturer (Applied Biosystems). The threshold cycle number (CT) value for UGT was normalized against GAPDH and calculated as $\Delta\text{CT} = \text{CT}_{\text{UGT}} - \text{CT}_{\text{GAPDH}}$. Relative UGT

mRNA expression was expressed as folds of UGT versus reference: $F = 2^{(\Delta CT)}$. All PCR reactions were performed in triplicate in three independent experiments.

Western Blot Analysis

Frozen tissues from normal donors and those with carcinomas were homogenized with a Polytron (Tekmar, Cincinnati, OH) in buffer containing 10 mM Tris-HCl, pH 7.8, 0.25 M sucrose, 6 N HCl, 1 mM EDTA, and protease inhibitors, followed by centrifugation at $17,500 \times g$ at 4 °C for 20 min. The supernatant was transferred to new centrifuge tubes and ultracentrifuged at $120,000 \times g$ at 4 °C for 60 min. The resulting pellet was suspended in buffer and protein concentration was determined using BioRad Protein Assay (Hercules, CA) according to the manufacturer's protocol. Microsomal protein (10 µg) from each tissue sample was suspended in phosphate-buffered saline (pH 7.4) containing 0.5 mmol/L dithiothreitol, boiled for 5 minutes, separated by SDS-PAGE on 10 % gels, and transferred to a nitrocellulose membrane following the method of Towbin [32]. The blotted membrane was blocked with 5 % fat-free dry milk for at least 2 h at room temperature and incubated with either anti-UGT1A to detect all UGT1A isoforms or anti-UGT2B7-peptide antibodies (1:500, Gentest, San Jose, CA) at 4 °C overnight. The membrane was then incubated for 1 h at room temperature with a horseradish peroxidase-conjugated goat anti-rabbit IgG at a 1:10,000 dilution (Gentest, San Jose, CA). The membrane was rinsed and immunoreactive protein was detected by enhanced chemiluminescence (ECL) for 1 min using SuperSignal West Femto reagents from Pierce (Rockford, IL). The membrane was then exposed to X-ray film at room temperature for 15 s to 1 min.

Glucuronidation Activity Assays

Frozen tissues from normal donors (n = 21) or those with carcinomas (n = 24) were suspended in phosphate-buffered saline (pH 7.4) containing 0.5 mmol/L dithiothreitol. The specimens were homogenized using a Polytron (Tekmar, Cincinnati, OH). Protein concentrations were determined using the BioRad Protein Assay Kit. UGT assays (30 µl total volume) contained 5 µl of buffer (1 M Tris, pH 8.0, 5 mM MgCl₂, 5 mM saccharolactone), 10 µg of protein, 250 µM unlabeled 4-OHE₁ or ³H-E₂ in 5% Brij-58 and 0.1 N NaOH. Reactions were initiated by adding 3 µl of either 50 mM unlabeled UDP-Glucuronic acid (UDP-GA), or [¹⁴C]UDP-GA (depending on if the substrate was radiolabeled), incubated at 37 °C for 1 h, terminated by adding 30 µl ethanol, and cooled on ice. Controls omitting either unlabeled UDP-GA or substrate were run with each assay.

Aliquots (40 µl) of each sample were then applied to the pre-absorbent layer of channeled silica gel TLC plates (Baker Si250-PA, 19C, VWR, Sugarland, TX). Products and un-reacted substrate were separated by development of the plates in chloroform-methanol-acetic acid-H₂O (65:25:2:4, v/v). Glucuronides present on silica gel were identified by autoradiography of the TLC plates for 3–5 days at –80 °C. Silica gel containing labeled glucuronides or in corresponding areas in control lanes was transferred to scintillation vials, and radioactivity was quantified by liquid scintillation counting (Packard TRI-CARB 2100TR, Perkin-Elmer) as previously described [33]. The position of glucuronidation (i.e., which hydroxyl group was glucuronidated) was not determined. Instead, we report the total glucuronidated substrate produced during the incubation.

Data Analysis

Prism IV software (GraphPad Software, San Diego, CA) was used for statistical analyses. Results from QRT-PCR and glucuronidation activity assays of E₂ and 4-OHE₁ are shown as the mean ± SEM. Data groups were analyzed using Student's t-tests to determine if there were significant differences between normal and cancer specimens. Differences were considered

significant at $p < 0.05$. Normal and cancer samples in which UGT mRNA expression was not detected by QRT-PCR were included for statistical analysis.

RESULTS

Quantification of UGT1A10 expression in normal and malignant breast tissues by QRT-PCR

QRT-PCR, as described in Materials and Methods, was used to analyze the mRNA expression levels of UGT1A10 in normal tissues ($n = 30$) and in breast cancer samples ($n = 31$) (Figure 1A). The expression levels were normalized using GAPDH as an internal control. The C_T values for GAPDH mRNA expression among all donors were consistent (data not shown), showing there was no significant variability in GAPDH mRNA expression. UGT1A10 mRNA was expressed in most tissue specimens; however, there was wide individual variation in transcript levels. In addition, UGT1A10 mRNA expression was significantly decreased by almost 4 fold in breast cancers in comparison to normal specimens (68 ± 26 for breast cancers vs. 252 ± 86 for normals, respectively; $p = 0.043$).

We also correlated UGT1A10 mRNA expression levels in tissue specimens with the ethnicity of the donors. Samples from African American women (normals, $n = 10$ and breast cancer, $n = 12$) displayed wide individual variation in expression of UGT1A10 mRNA (Figure 1B). Despite this, expression of UGT1A10 in breast cancer specimens was significantly (7 fold) reduced in comparison to normal specimens (57 ± 35 for breast cancers vs. 397 ± 152 for normals, $p = 0.029$). In contrast, for Caucasian women, expression of UGT1A10 in normal specimens ($n = 20$) was only 2 fold higher than breast cancer specimens ($n = 19$) (Figure 1C).

Quantification of UGT2B7 expression in normal and malignant breast tissues by QRT-PCR

QRT-PCR was repeated for UGT2B7 mRNA expression in normals ($n = 28$) and breast cancer specimens ($n = 29$) (Figure 2A). As with UGT1A10, UGT2B7 displayed broad individual variations in mRNA expression. Furthermore, UGT2B7 mRNA expression was significantly decreased by more than 8 fold in breast cancer specimens in comparison to normal specimens (1.4 ± 0.69 for breast cancers vs. 12 ± 4.1 for normals, $p = 0.01$). When ethnicity was considered, normals ($n = 8$) and breast cancer specimens ($n = 11$) from African American women also displayed broad individual variation in expression of UGT2B7 mRNA (Figure 2B) and UGT2B7 expression in normal specimens was 7 fold higher than in breast cancer specimens (0.4 ± 0.2 for breast cancers vs. 3.2 ± 2 for normals, $p = 0.065$). In Caucasian women, expression of UGT2B7 in normal specimens ($n = 20$) was significantly higher, almost 13 fold (1.1 ± 0.5 for breast cancers vs. 14 ± 5.6 for normals; $p = 0.049$), than breast cancer specimens ($n = 17$) (Figure 2C).

Differential UGT activity in normal and malignant breast tissues

Total protein from homogenized breast tissues was used in UGT activity assays to determine functional differences between carcinoma and normal breast tissues. UGT activity toward E_2 and 4-OHE₁, the genotoxic metabolite of estrogen, were compared in breast carcinoma ($n = 23$) and normal ($n = 21$) tissues (Figure 3A and 3B). Glucuronidation of E_2 was reduced 2 fold in breast carcinomas compared to normal breast samples (14.9 ± 4.8 vs. 33.7 ± 12 pmol/mg protein/min for carcinoma and normal tissues, respectively), while the same activities for 4-OHE₁ were reduced almost 4 fold in breast carcinomas as compared to normal breast tissues (30 ± 15 vs. 106 ± 31 pmol/mg protein/min for carcinoma and normal tissues, respectively). Interestingly, normal breast specimens conjugated the genotoxic 4-OHE₁ with more than 3 fold higher activity than E_2 (106 ± 31 vs. 33.7 ± 12 pmol/mg protein/min for 4-OHE₁ and E_2 , respectively).

Expression of UGT1A10 and UGT2B7 mRNA, protein, and enzymatic activity in normal and malignant breast tissues from the same donor

Expression of UGT1A10 and UGT2B7 gene products was analyzed by QRT-PCR in three normal/carcinoma breast tissue sample pairs from the same individual (Figure 4A). Comparison of the three sets of breast carcinomas with adjacent normal breast epithelia from the same donor revealed substantial differential and individual regulation for the UGT1A10 and UGT2B7 transcripts. The relative mRNA expression levels of UGT1A10 in sample 1, tumor (T1) is 3 folds lower than the adjacent sample 1, normal (N1). However, UGT2B7 mRNA levels are 6 fold higher in tumor than in the normal. Expression of UGT1A and UGT2B7 proteins in sample 1 by Western blot revealed that UGT1A and protein levels were differentially down regulated in breast carcinomas in comparison to normal breast epithelia; however, the protein expression of UGT2B7 in the tumor is much higher than the adjacent normal sample pair (Figure 4B). In agreement with the QRT-PCR and Western blot analysis, the catalytic activity exhibited by sample 1 revealed slightly higher conjugating activity for 4-OHE₁ in the tumor than in the adjacent normal sample pairs (7.1 pmol/mg protein/min for sample 1, tumor vs. 5.4 pmol/mg protein/min for normal) (Figure 4C). The relative mRNA expression levels of UGT1A10 in sample 2, tumor (T2) is 2 folds lower than in the adjacent sample 2, normal (N2). UGT2B7 mRNA levels are also 5 fold lower in tumor than in the normal (Figure 4A). Expression of UGT1A and UGT2B7 proteins in sample 2 were differentially down regulated in breast carcinomas in comparison to normal breast epithelia (Figure 4B), and the catalytic activity exhibited by sample 2 revealed higher conjugating activity for 4-OHE₁ in the normal (3.4 pmol/mg protein/min) than in the adjacent tumor sample pairs which was not detected (Figure 4C). The relative mRNA expression levels of UGT1A10 and UGT2B7 in sample 3, normal (N3) were much higher than in the adjacent tumor (T3), which was not detected by QRT-PCR (Figure 4A). Expression of UGT1A and UGT2B7 proteins in sample 3 were differentially down-regulated in breast carcinomas in comparison to normal breast epithelia (Figure 4B), and the catalytic activity exhibited by sample 3 revealed much higher conjugating activity for 4-OHE₁ in the normal (77 pmol/mg protein/min) than in the adjacent tumor sample pairs (17 pmol/mg protein/min) (Figure 4C).

DISCUSSION

Glucuronidation of estrogens, catalyzed by UGTs, is an important process in human metabolic catabolism, since two native estrogens, E₁ and E₂, and at least one oxidative metabolite of estrogen, 4-OHE₁, are associated with breast carcinogenesis. In a previous report, seven recombinant human UGTs, UGT1A1, 1A3, 1A4, 1A6, 1A7, 1A8, 1A9, 1A10, and 2B7, were identified as being involved in the biotransformation of native estrogen and/or their oxidative derivatives [20]. Of those UGTs, UGT1A10 exhibited the highest conjugative activities towards all the estrogens examined, including the genotoxic 4-OHE₁. UGT2B7 was also found to have high conjugating activity towards 4-OHE₁.

Although most of the UGT cDNAs have been cloned and characterized in hepatic and extrahepatic tissues [34–36], the regulation and function of UGT1A10 and UGT2B7 within estrogen target tissues including the breast is less well established [30]. One study identified UGT2B7 expression in the ductal epithelial cells of the breast [30]. Furthermore, it was demonstrated that UGT2B7 expression in the ductal epithelial cells of the mammary glands and the formation of 4-OHE₁ glucuronides was down-regulated or abolished in invasive breast carcinomas [30]. The marked reduction in expression of UGT2B7 in invasive breast cancers compared to normal breast parenchyma was suggested to be due to an anticarcinogenic function for the enzyme [30].

In the current study, we provide novel evidence of UGT1A10 mRNA expression in the breast for the first time. In a total of 61 normal and malignant human breast specimens from African

American and Caucasian women, UGT1A10 and UGT2B7 mRNA displayed significant individual differences in expression. Moreover, UGT1A10 and UGT2B7 transcripts were differential down-regulated among cancer specimens. The significant individual down-regulation in the level of expression of UGT1A10 mRNA in breast carcinomas obtained from African American women is worth noting. This observation might be due to higher circulating E₂ and 4-OHE₁ levels in the breast of African American women, as well as in their exposure to xenobiotics and drugs [30,37–39]. In support of this finding, it has been suggested that UGTs might play an essential role in controlling the steady-state concentrations of ligands for nuclear receptor [40]. This property of UGTs is especially important to ensure complete detoxification of potentially harmful compounds, including estrogens, which could otherwise accumulate in the body. Recently published studies from our laboratory demonstrated for the first time that UGT1A10 is regulated by E₂ in a dose-dependent manner and that its regulation is mediated *via* the ER [41]. Furthermore, UGT1A10 mRNA expression was induced by E₂ and then down-regulated by E₂ at pharmacological concentrations in the MCF-7 ER-positive breast cancer cell line. Although the molecular mechanism of UGT1A10 regulation in the breast is unknown, it is interesting to hypothesize that the significant down-regulation in UGT1A10 mRNA expression observed in African American women might be due to the accumulation of pharmacological concentrations of E₂ in the cells of the breast. This accumulation might have a significant moderating effect on E₂ availability for ER and estrogen clearance, thereby promoting the signaling of E₂ in breast cancer cells, which could stimulate the growth and proliferation of the tumor.

The findings presented also show that in normal breast and paired breast cancers, there occurs significant individual regulation of UGT1A10 and UGT2B7 gene products and UGT1A and UGT2B7 proteins. Interestingly, UGT2B7 mRNA was up-regulated in a cancer specimen from one donor as compared to the adjacent normal tissue. This study also included E₂ and the genotoxic 4-OHE₁, which was glucuronidated at significantly lower rates in breast carcinomas in comparison to normal specimens. This finding suggests that glucuronidation is essential for controlling the levels of E₂ in the breast and for the protection against oxidation to genotoxic 4-OH-E₁ molecules. Furthermore, the interindividual differences in UGT1A10 and UGT2B7 expression and activity may be due to polymorphic regulation of these UGT genes that have been extensively researched [11,25,42,43]. As a consequence, the interindividual differences in UGT expression and activity could potentially modify the cellular defense potential of the breast against free oxygen radicals produced during redox cycling between the semiquinone and quinone intermediates of the catecholestrogens, thereby affecting breast cancer predisposition [30,44]. Collectively, the data provides compelling evidence for the individual regulation of UGT1A10 and UGT2B7 genes.

In conclusion, this novel work represents the first quantitative identification of UGT1A10 expression in normal breast tissues and breast carcinomas from African American and Caucasian women. This study also represents the first identification of racial/ethnic differences in UGT1A10 and UGT2B7 expression in human breast tissues. The ability of most of the examined normal breast specimens, but not invasive breast carcinomas, to glucuronidate the genotoxic 4-OHE₁ further supports a biological, protective role for the UGT pathway in the breast. Defining the molecular mechanism that results in the individual differences of UGT1A10 and UGT2B7 expression in the breast will provide novel insight into the role UGTs might play in protecting the breast from the carcinogenic effects of estrogens. Although this study has limitations including the small sample size and the lack of a specific antibody for UGT1A10, this study was still able to determine the statistical significance of UGT1A10 mRNA expression and glucuronidation activity in the breast for the first time.

ACKNOWLEDGMENTS

Grant support: This work was supported in part by National Institute of Health grant DK60109 to A. Radominska-Pandya. A. Starlard-Davenport is the recipient of a minority supplement award from the National Institute of Health.

We thank Joanna M. Little, Stacie M. Bratton, and George J. Hammons, PhD, for their expertise.

The views presented in this article do not necessarily reflect those of the US Food and Drug Administration.

REFERENCES

1. Dutton, GJ. Glucuronidation of Drugs and Other Compounds. Boca Raton, FL: CRC Press Inc.; 1980.
2. Bock KW, Lilienblum W, Fischer G, et al. The role of conjugation reactions in detoxication. *Arch Toxicol* 1987;60(1-3):22-29. [PubMed: 3304211]
3. Formelli F, Barua AB, Olson JA. Bioactivities of N-(4-hydroxyphenyl) retinamide and retinoyl beta-glucuronide. *FASEB Journal* 1996;10(9):1014-1024. [PubMed: 8801162]
4. Vore M, Montgomery C, Meyers M. Steroid D-ring glucuronides: characterization of a new class of cholestatic agents. *Drug Metab Rev* 1983;14(5):1005-1019. [PubMed: 6653400]
5. Olson JA, Moon RC, Anders MW, et al. Enhancement of biological activity by conjugation reactions. *Journal of Nutrition* 1992;122(3 Suppl):615-624. [PubMed: 1531853]
6. Abbott FV, Palmour RM. Morphine-6-glucuronide: analgesic effects and receptor binding profile in rats. *Life Sci* 1988;43(21):1685-1695. [PubMed: 2848167]
7. Munzel PA, Bookjans G, Mehner G, et al. Tissue-specific 2,3,7,8-tetrachlorodibenzo-p-dioxin-inducible expression of human UDP-glucuronosyltransferase UGT1A6. *Arch Biochem Biophys* 1996;335(1):205-210. [PubMed: 8914852]
8. Burchell B, Brierley CH, Monaghan G, Clarke DJ. The structure and function of the UDP-glucuronosyltransferase gene family. *Adv Pharmacol* 1998;42:335-338. [PubMed: 9327909]
9. Harding D, Jeremiah SJ, Povey S, Burchell B. Chromosomal mapping of a human phenol UDP-glucuronosyltransferase, GNT1. *Ann Hum Genet* 1990;54(Pt 1):17-21. [PubMed: 2108603]
10. Tukey RH, Strassburg CP. Human UDP-glucuronosyltransferases: metabolism, expression, and disease. *Annu Rev Pharmacol Toxicol* 2000;40:581-616. [PubMed: 10836148]
11. Strassburg CP, Nguyen N, Manns MP, Tukey RH. Polymorphic expression of the UDP-glucuronosyltransferase UGT1A gene locus in human gastric epithelium. *Mol Pharmacol* 1998;54(4):647-654. [PubMed: 9765507]
12. Strassburg CP, Manns MP, Tukey RH. Expression of the UDP-glucuronosyltransferase 1A locus in human colon. Identification and characterization of the novel extrahepatic UGT1A8. *J Biol Chem* 1998;273(15):8719-8726. [PubMed: 9535849]
13. Mojarrabi B, Mackenzie PI. Characterization of two UDP glucuronosyltransferases that are predominantly expressed in human colon. *Biochem Biophys Res Commun* 1998;247(3):704-709. [PubMed: 9647757]
14. Lepine J, Bernard O, Plante M, et al. Specificity and regioselectivity of the conjugation of estradiol, estrone, and their catecholesterogen and methoxyestrogen metabolites by human uridine diphospho-glucuronosyltransferases expressed in endometrium. *J Clin Endocrinol Metab* 2004;89(10):5222-5232. [PubMed: 15472229]
15. Li JJ, Li SA. Estrogen carcinogenesis in Syrian hamster tissues: role of metabolism. *Fed Proc* 1987;46(5):1858-1863. [PubMed: 3030825]
16. Feigelson HS, Henderson BE. Estrogens and breast cancer. *Carcinogenesis* 1996;17(11):2279-2284. [PubMed: 8968038]
17. Cavalieri EL, Stack DE, Devanesan PD, et al. Molecular origin of cancer: catechol estrogen-3,4-quinones as endogenous tumor initiators. *Proc Natl Acad Sci U S A* 1997;94(20):10937-10942. [PubMed: 9380738]
18. Yager JD. Endogenous estrogens as carcinogens through metabolic activation. *J Natl Cancer Inst Monogr* 2000;(27):67-73. [PubMed: 10963620]
19. Stack DE, Byun J, Gross ML, et al. Molecular characteristics of catechol estrogen quinones in reactions with deoxyribonucleosides. *Chem Res Toxicol* 1996;9(5):851-859. [PubMed: 8828920]

20. Starlard-Davenport A, Xiong Y, Bratton S, et al. Phenylalanine(90) and phenylalanine(93) are crucial amino acids within the estrogen binding site of the human UDP-glucuronosyltransferase 1A10. *Steroids* 2007;72(1):85–94. [PubMed: 17174996]
21. Basu NK, Kubota S, Meselhy MR, et al. Gastrointestinally distributed UDP-glucuronosyltransferase 1A10, which metabolizes estrogens and nonsteroidal anti-inflammatory drugs, depends upon phosphorylation. *J Biol Chem* 2000;275(27):28320–28329. [PubMed: 15117964]
22. Gall WE, Zawada G, Mojarrabi B, et al. Differential glucuronidation of bile acids, androgens and estrogens by human UGT1A3 and 2B7. *J Steroid Biochem Mol Biol* 1999;70(1–3):101–108. [PubMed: 10529008]
23. Guillemette C, Millikan RC, Newman B, Housman DE. Genetic polymorphisms in uridine diphosphate-glucuronosyltransferase 1A1 and association with breast cancer among African Americans. *Cancer Res* 2000;60(4):950–956. [PubMed: 10706110]
24. Chouinard S, Tessier M, Vernouillet G, et al. Inactivation of the pure antiestrogen fulvestrant and other synthetic estrogen molecules by UDP-glucuronosyltransferase 1A enzymes expressed in breast tissue. *Mol Pharmacol* 2006;69(3):908–920. [PubMed: 16339389]
25. Thibaudeau J, Lepine J, Tojcic J, et al. Characterization of common UGT1A8, UGT1A9, and UGT2B7 variants with different capacities to inactivate mutagenic 4-hydroxylated metabolites of estradiol and estrone. *Cancer Res* 2006;66(1):125–133. [PubMed: 16397224]
26. Albert C, Vallee M, Beaudry G, et al. The monkey and human uridine diphosphate-glucuronosyltransferase UGT1A9, expressed in steroid target tissues, are estrogen-conjugating enzymes. *Endocrinology* 1999;140(7):3292–3302. [PubMed: 10385426]
27. Turgeon D, Carrier JS, Levesque E, et al. Relative enzymatic activity, protein stability, and tissue distribution of human steroid-metabolizing UGT2B subfamily members. *Endocrinology* 2001;142(2):778–787. [PubMed: 11159850]
28. Harrington WR, Sengupta S, Katzenellenbogen BS. Estrogen regulation of the glucuronidation enzyme UGT2B15 in estrogen receptor-positive breast cancer cells. *Endocrinology* 2006;147(8):3843–3850. [PubMed: 16690804]
29. Levesque E, Turgeon D, Carrier JS, et al. Isolation and characterization of the UGT2B28 cDNA encoding a novel human steroid conjugating UDP-glucuronosyltransferase. *Biochemistry* 2001;40(13):3869–3881. [PubMed: 11300766]
30. Gestl SA, Green MD, Shearer DA, et al. Expression of UGT2B7, a UDP-glucuronosyltransferase implicated in the metabolism of 4-hydroxyestrone and all-trans retinoic acid, in normal human breast parenchyma and in invasive and in situ breast cancers. *Am J Pathol* 2002;160(4):1467–1479. [PubMed: 11943730]
31. Congiu M, Mashford ML, Slavin JL, Desmond PV. UDP glucuronosyltransferase mRNA levels in human liver disease. *Drug Metab Dispos* 2002;30(2):129–134. [PubMed: 11792680]
32. Towbin H, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci U S A* 1979;76(9):4350–4354. [PubMed: 388439]
33. Little JM, Williams L, Xu J, Radomska-Pandya A. Glucuronidation of the dietary fatty acids, phytanic acid and docosahexaenoic acid, by human UDP-glucuronosyltransferases. *Drug Metab Dispos* 2002;30(5):531–533. [PubMed: 11950783]
34. Ritter JK, Crawford JM, Owens IS. Cloning of two human liver bilirubin UDP-glucuronosyltransferase cDNAs with expression in COS-1 cells. *J Biol Chem* 1991;266(2):1043–1047. [PubMed: 1898728]
35. Wooster R, Sutherland L, Ebner T, et al. Cloning and stable expression of a new member of the human liver phenol/bilirubin: UDP-glucuronosyltransferase cDNA family. *Biochem J* 1991;278(Pt 2):465–469. [PubMed: 1910331]
36. Mojarrabi B, Butler R, Mackenzie PI. cDNA cloning and characterization of the human UDP glucuronosyltransferase, UGT1A3. *Biochem Biophys Res Commun* 1996;225(3):785–790. [PubMed: 8780690]
37. Setiawan VW, Haiman CA, Stanczyk FZ, et al. Racial/ethnic differences in postmenopausal endogenous hormones: the multiethnic cohort study. *Cancer Epidemiol Biomarkers Prev* 2006;15(10):1849–1855. [PubMed: 17035391]

38. Cunningham JE, Butler WM. Racial disparities in female breast cancer in South Carolina: clinical evidence for a biological basis. *Breast Cancer Res Treat* 2004;88(2):161–176. [PubMed: 15564799]
39. Woodward WA, Huang EH, McNeese MD, et al. African-American race is associated with a poorer overall survival rate for breast cancer patients treated with mastectomy and doxorubicin-based chemotherapy. *Cancer* 2006;107(11):2662–2668. [PubMed: 17061247]
40. Radomska-Pandya A, Little JM, Czernik PJ. Human UDP-glucuronosyltransferase 2B7. *Curr Drug Metab* 2001;2(3):283–298. [PubMed: 11513331]
41. Starlard-Davenport A, Lyn-Cook B, Radomska-Pandya A. Novel identification of UDP-glucuronosyltransferase 1A10 as an estrogen-regulated target gene. *Steroids*. 2007
42. Guillemette C, De Vivo I, Hankinson SE, et al. Association of genetic polymorphisms in UGT1A1 with breast cancer and plasma hormone levels. *Cancer Epidemiol Biomarkers Prev* 2001;10(6):711–714. [PubMed: 11401924]
43. Fujita VS, Thiele DJ, Coon MJ. Expression of alcohol-inducible rabbit liver cytochrome P-450 3a (P-450IIE1) in *Saccharomyces cerevisiae* with the copper-inducible CUP1 promoter. *DNA. Cell. Biol* 1990;9:111–118.
44. Guillemette C, Belanger A, Lepine J. Metabolic inactivation of estrogens in breast tissue by UDP-glucuronosyltransferase enzymes: an overview. *Breast Cancer Res* 2004;6(6):246–254. [PubMed: 15535854]

Abbreviations

UDP-glucuronosyltransferase, UGT.

Figure 1.

Characterization of UGT1A10 mRNA expression in normal and malignant breast tissues. (A) Total RNA isolated from normal (n = 30) and breast carcinoma (n = 31) tissues from African American and Caucasian women was reverse-transcribed, pooled into the two respective ethnic groups, and quantified by QRT-PCR for UGT1A10 mRNA levels. (B) Normal (n = 10) and breast carcinomas (n = 12) from African American women were examined by QRT-PCR for UGT1A10 mRNA levels. (C) Normal (n = 20) and breast cancer specimens (n = 19) from Caucasian women were examined as in 2C. $P < 0.05$ is considered statistically significant. Normal and cancer samples in which UGT1A10 mRNA expression was not detected by QRT-PCR were included for statistical analysis.

Figure 2.

Characterization of UGT2B7 mRNA expression in normal and malignant breast tissues. (A) Total RNA isolated from normal (n = 28) and breast carcinoma (n = 28) tissues from African American and Caucasian women was reverse-transcribed, pooled into the two respective ethnic groups, and quantified by QRT-PCR for UGT2B7 mRNA levels. (B) Normal (n = 8) and breast carcinomas (n = 11) from African American women were examined by QRT-PCR for UGT2B7 mRNA levels. (C) Normal (n = 20) and breast cancer specimens (n = 17) from Caucasian women were examined as in 2C. $P < 0.05$ is considered statistically significant. Normal and cancer samples in which UGT2B7 mRNA expression was not detected by QRT-PCR were included for statistical analysis.

Figure 3.

UDP-glucuronosyltransferase activity of tissue obtained from normal and cancer breast tissue specimens. Ten micrograms of total protein isolated from normal breast tissue (n = 21) and breast carcinomas (n = 24) from African American and Caucasian women were pooled to assess their ability to glucuronidate E₂ and 4-OHE₁ (Figure 4A and 4B). Glucuronides formed from the reaction were separated by thin layer chromatography and subjected to scintillation counting. Statistical significance of differences in glucuronidation of E₂ and 4-OHE₁ by tissue from non-malignant and malignant breast tissue was determined by Student's *t*-test. *P* < 0.05 is considered statistically significant. Normal and cancer samples that were not detected by glucuronidation activity assays were included for statistical analysis.

Figure 4.

Expression of UGT1A10 and UGT2B7 mRNA, protein, and enzymatic activity in normal and malignant human breast tissues from the same donor. Normal tissues are represented by white bars, tumor tissues by grey bars. (A) QRT-PCR analysis, as described in "Methods and Materials" was used to determine UGT1A10 and UGT2B7 mRNA levels in breast carcinomas and adjacent normal tissues from the same donor in three different individuals. The real-time PCR results were standardized against GAPDH, and relative arbitrary units were calculated for each gene. (B) Protein expression was determined by Western blot as described in "Methods and Materials" using protein (10 μ g) from adjacent normal and tumor tissue pairs and a UGT1A antibody, which detects all UGT1A isoforms, and a specific UGT2B7 antibody were used to identify expressed protein in the tissues. Calnexin protein levels were also monitored to assess protein loading. (C) UGT activities toward 4-OHE₁ were determined as described in "Methods and Materials" using protein (10 μ g) from normal and adjacent tumor tissue pairs. Activity is expressed in pmol/mg protein/min and each value represents the mean $\pm$ SEM from two independent experiments performed in duplicate. ND indicates not detectable.

Table 1

Characteristics of breast specimens used in these studies.

Ethnicity	Histology	Mean Age $\pm$ SEM	Number of Specimens
African Americans	Normal	43.7 $\pm$ 21.7	10
	ID	50.9 $\pm$ 17.3	10
	IL	49.0	1
	ID and IL	80.0	1
Caucasians	Normal	50.8 $\pm$ 23.4	20
	ID	63.9 $\pm$ 19.6	17
	IL	78.0 $\pm$ 15.6	2
* Breast carcinoma and adjacent normal		43.5 $\pm$ 18.6	6

* Carcinoma and adjacent normal breast tissue from the same donor

ID: Infiltrating Ductal Carcinoma

IL: Infiltrating Lobular Carcinoma

Table 2

Genes	Primer Sequence (F = forward; R = reverse)	Amplicon Size (bp)
UGT1A1	F: 5' TTTGTCTGGCTGTTCCCACT R: 5' GAAGGTCATGTGATCTGAATGAGA	251
UGT1A3	F: 5' ATGTGCTGGGCCACACTTCAACT R: 5' TCATTATGAGTAGCTCCACACAA	130
UGT1A4	F: 5' ACGCTGGGCTACACTCAAGG R: 5' TCATTATGCAGTAGCTCCACACA	128
UGT1A6	F: 5' CTTTTCACAGACCCAGCCTTAC R: 5' TATCCACATCTCTCTTGAGGACAG	289
UGT1A8	F: 5' GGTCTTCGCCAGGGGAATAGG R: 5' GTTCGCAAGATTCGATGGTCG	235
UGT1A9	F: 5' GAGGAACATTTATTATGCCACCG R: 5' GCAACAACCAAATTGATGTGTG	118
UGT1A10	F: 5' CCTCTTTCCTATGTCCCAATGA R: 5' GCAACAACCAAATTGATGTGTG	205
UGT2B4	F: 5' CTGTTGTTATGTCAGAAC R: 5' TTCCTAGAACTTCACTGTAG	278
UGT2B7	F: 5' CCTGCCTAAGGAAATGGAAGAC R: 5' AACCTTTTGTGGGATCTGGGCC	232
UGT2B15	F: 5' GTGTTGGGAATATTATGACTACAGTAAC R: 5' GGGTATGTTAAATAGTTCAGCCAGT	141
UGT2B17	F: 5' TGACTTTTGGTTTCAAGC R: 5' TTCCATTTCTTCTTAGGCAA	220
GAPDH	F: 5' ACCCACTCCTCCACCTTTG R: 5' CTCTTGTGCTCTTGCTGGG	195