Androgen Receptor Signals Regulate UDP-Glucuronosyltransferases in the Urinary Bladder: A Potential Mechanism of Androgen-Induced Bladder Carcinogenesis

Koji Izumi, Yichun Zheng, Jong-Wei Hsu, Chawnshang Chang, and Hiroshi Miyamoto*
Department of Pathology and Laboratory Medicine, University of Rochester Medical Center, Rochester, New York

UDP-glucuronosyltransferases (UGTs), major phase II drug metabolism enzymes, play an important role in urinary bladder cancer initiation by detoxifying carcinogens. We aimed to determine if androgens regulate UGT expression via the androgen receptor (AR) pathway in the bladder. Real-time reverse transcription-polymerase chain reaction and Western blot analyses were used to assess UGT1A levels in the normal urothelium SVHUC cell line stably expressed with AR and in bladder tissues from AR knockout (ARKO) and castrated male mice. Immunohistochemistry was also performed in radical cystectomy specimens. Dihydrotestosterone (DHT) treatment in SVHUC-AR reduced mRNA expression of all the UGT1A subtypes (19–75% decrease), and hydroxyflutamide antagonized the DHT effects. In contrast, DHT showed only marginal effects on UGT1A expression in SVHUC-Vector. Of note were higher expression levels of UGT1A1 in SVHUC-Vector than in SVHUC-AR. In ARKO mice, all the Ugta subtypes were up-regulated, compared to wild-type littermates. In wild-type male mice, castration increased the expression of Ugta18, Ugta19, and Ugta10. Additionally, wild-type female mice had higher levels of Ugta1 than wild-type males. Immunohistochemical studies showed strong (3+) UGT1A staining in 11/24 (46%) cancer tissues, which was significantly lower than in corresponding benign tissues (17/18 (94%) cases (P = 0.0009)). These results suggest that androgen-mediated AR signals promote bladder carcinogenesis by down-regulating the expression of UGTs in the bladder.

INTRODUCTION

Urinary bladder cancer is at least three times more common among males than females worldwide [1]. Excessive exposure to carcinogens, such as cigarette smoke and industrial chemicals, has been suggested to be a cause of higher incidence of bladder cancer in men. However, after controlling for these carcinogenic factors, men still have a substantially higher risk of bladder cancer than women [1,2]. We recently showed molecular evidence suggesting that androgen receptor (AR) signaling pathway promotes bladder carcinogenesis as well as cancer progression [3]. Castrated male and wild-type female mice had a lower incidence of bladder cancer induced by a chemical carcinogen, N-butyln-(4-hydroxybutyl)nitrosamine (BBN), than wild-type male mice. In addition, AR knockout (ARKO) in male and female mice completely prevented bladder cancer development.

Industrial chemicals, such as aromatic amines, are well-known bladder carcinogens. They can be glucuronidated in the liver and excreted either by the biliary system into the intestine or via the blood into the urinary system [4]. In the bladder, their glucuronides may accumulate in the bladder epithelium. Then, these accumulated aromatic amines undergo further metabolism by peroxidation and/or O-acetylation to form DNA adducts that may initiate bladder carcinogenesis [5]. 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) is one of the most potent and abundant procarcinogens found in tobacco and tobacco smoke. NNK is rapidly metabolized to its carbonyl

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reduction, 4-(methylnitrosoamo)-1-(3-pyridyl)-1-butanol (NNAL). NNAL can be detoxified by forming glucuronidated NNAL, which is readily excreted in the urine. Indeed, the level of urinary NNAL is used as a biomarker for environmental tobacco smoke [6] and has been reported to correlate with smoking status in bladder cancer patients [7].

UDP-glucuronosyltransferases (UGTs) belong to a superfamily of major phase II drug metabolism enzymes that catalyze the glucuronidation of numerous endobiotics and xenobiotics. All known human UGTs are divided into three subfamilies, UGT1A, UGT2A, and UGT2B, based on gene sequence homology [8]. There are 13 subtypes of human UGT1A gene, located on chromosome 2q37, consisting of 13 individual promoters and different first exons. Each exon 1 is combined to four common exons by alternative splicing, generating four pseudogenes and nine functional proteins (UGT1A1, UGT1A3, UGT1A4, UGT1A5, UGT1A6, UGT1A7, UGT1A8, UGT1A9, and UGT1A10). In contrast to the UGT1A, the UGT2 subfamily consists of individual gene cluster. In mouse, the genes are described in lower case after first letter. Mouse Ugt1a locus on chromosome 1C5/D contains 14 different first exons and a shared set of exons 2–5, generating five pseudogenes and nine functional genes (Ugt1a1, Ugt1a2, Ugt1a5, Ugt1a6a, Ugt1a6b, Ugt1a7c, Ugt1a8, Ugt1a9, and Ugt1a10) [9]. There is no clear consensus about the functional homology between human UGT and mouse Ugt.

The UGT1A superfamily, rather than UGT2A and UGT2B, generally plays a more important role in metabolism of aromatic amines. In the liver, each of UGT1A1, UGT1A4, or UGT1A9 is able to metabolize all of the aromatic amines tested, but UGT1A4 and UGT1A9 exhibit higher rates of metabolism [10]. UGT1A4 and UGT1A9 have also been shown to possess NNAL glucuronidation activity [11]. Although the liver is considered the most important organ for metabolism, including glucuronidation, UGTs are also expressed in some extrahepatic tissues, such as the gastrointestinal tract [12], kidney [13], and aerodigestive tract [14]. A recent study, using various human tissues, revealed that normal bladder expresses all the UGT subtypes except UGT2B17 [15]. Additionally, down-regulation of UGT1A expression was observed in several bladder cancer tissue samples, compared to normal urothelium [16,17].

Thus, UGT1As are likely key enzymes involved in detoxification of major bladder carcinogens. Interestingly, UGT1A has been identified as an androgen responsive gene in a prostate cancer cell line [18]. The purpose of this study was to investigate the relationship between AR signals and UGT1A/Ugt1a expression in the bladder. Alterations of UGT expression in bladder urothelium could be underlying mechanisms responsible for bladder carcinogenesis mediated via the AR signaling pathway.

MATERIALS AND METHODS

Cell Culture and Chemicals

Human urothelium cell line SVHUC and human embryonic kidney cell line 293T (both obtained from the American Type Culture Collection, Manassas, VA) were maintained in appropriate media (Mediatech, Manassas, VA; Kaihn’s Modification of Ham’s F-12 for SVHUC; Dulbecco’s modified Eagle’s medium for 293T) supplemented with 10% fetal bovine serum (FBS) at 37°C in humidified atmosphere of 5% CO2. Cells were cultured in phenol-red free medium supplemented with 5% charcoal-stripped FBS at least 18 h before experimental treatment. We obtained dihydrotestosterone (DHT) from Sigma (St. Louis, MO) and hydroxyflutamide (HF) from Schering (Kenilworth, NJ).

Stable Cell Line With AR

To establish a cell line stably expressing the AR, a lentivirus vector pWPI-AR/pWPI-control, psPAX2, and pMD2.G were first co-transfected into 293T cells using GeneJuice transfection reagent (Novagen, Gibbstown, NJ). Forty-eight hours after transfection, SVHUC cells were cultured in the presence of viral supernatant containing 8 μg/mL polybrene (Millipore, Billerica, MA) for 6 h. Flow cytometry was used to obtain pure SVHUC overexpressing AR (SVHUC-AR) or vector only (SVHUC-V).

Reporter Gene Assay

SVHUC cells at a density of 50–60% confluence in 24-well plates were co-transfected with 250 ng of MMTV-luc reporter plasmid DNA and 2.5 ng of pRL-TK-luc plasmid DNA, using GeneJuice. Six hours after transfection, the medium was replaced with medium supplemented with 5% charcoal-stripped FBS in the presence of ligands (DHT, HF, or both) for 24 h. Cells were harvested, lysed, and assayed for luciferase activity determined using a Dual-Luciferase Reporter Assay kit (Promega, Madison, WI) and luminometer (TD-20/20, Turner Biosystems, Sunnyvale, CA).

Reverse Transcription (RT) and Real-Time Polymerase Chain Reaction (PCR)

Total RNA (1.0 μg) isolated using TRIzol (Invitrogen, Carlsbad, CA) was reverse transcribed using 1 μmol/L oligo (dT) primers and four units of Omniscript reverse transcriptase (Qiagen, Valencia, CA) in a total volume of 20 μL. Real-time PCR was then performed in 15 μL system by using SYBR GreenER qPCR SuperMix for iCycler (Invitrogen), as described previously [3]. The primer sequences...
Table 1. Sequences of Primers Used for Real-Time RT-PCR Analysis

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<th>Primer</th>
<th>Sense</th>
<th>Anti-sense</th>
<th>Amplicon size</th>
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Molecular Carcinogenesis
and 15 ≥pT3 tumors, as well as 12 node-negative and 12 node-positive tumors. Immunohistochemical staining was then performed, as described previously [19,21] with minor modifications. Briefly, TMA sections (4 μm thick) were deparaffinized in xylene, rehydrated in a graded ethanol series, and incubated in 3% hydrogen peroxide to block endogenous peroxidase. Slides were incubated overnight at 4°C with anti-AR (N20) antibody (diluted 1:100) and anti-UGT1A (H300) antibody (diluted 1:100; Santa Cruz Biotechnology). We then incubated the samples with a broad spectrum secondary antibody (Invitrogen). Slides were incubated with diaminobenzidine (Invitrogen), and finally counterstained with hematoxylin. These stains were manually scored by one pathologist (H.M.) blinded to patient identity. German Immunoreactive Score (0–12) was calculated, separately in benign and malignant tissues, by multiplying the percentage of immunoreactive cells (0% = 0; 1–10% = 1; 11–50% = 2; 51–80% = 3; 81–100% = 4) by staining intensity (negative = 0; weak = 1; moderate = 2; strong = 3). Scores were considered negative (--; 0–1), weakly positive (1+; 2–4), moderately positive (2++; 6–8), and strongly positive (3++; 9–12).

Statistical Analysis
Student's t-test was used to analyze differences in mRNA expression levels of UGT/Ugt between the two groups. Fisher's exact test and the chi-square test were used to analyze differences in UGT1A expression in bladder TMA. Survival rates in patients were calculated by the Kaplan–Meier method and comparison was made by log-rank test. P values less than 0.05 were considered to be statistically significant.

RESULTS
Expression and Transcriptional Activity of AR in SVHUC Cells
Because our preliminary study indicated human normal urothelium cell line SVHUC lacked AR, a human full-length wild-type AR was stably expressed in the cells by lentivirus. Then, we tested AR expression in SVHUC-AR and SVHUC-V with treatment of DHT and/or antiandrogen HF. As shown in Figure 1A, strong expression of AR protein was confirmed in SVHUC-AR, but not in SVHUC-V. AR expression in SVHUC-AR was enhanced by DHT treatment and antagonized by HF, which by itself showed marginal effects. Luciferase activity was also determined in these cell extracts with transfection of a plasmid containing an androgen response element as a reporter of AR-mediated transcriptional activity. As shown in Figure 1B, DHT treatment increased luciferase activity by 44-fold over mock treatment in SVHUC-AR, and HF showing only marginal activity clearly blocked the DHT effect. In SVHUC-V, DHT and HF showed only marginal effects on AR transcriptional activity. These data indicate that SVHUC-AR, but not SVHUC-V, possesses an active AR.

Androgen/AR-Mediated Down-Regulation of UGT1A in SVHUC Cells
We first tested mRNA expression of all the UGT1A subtypes in SVHUC-AR in the presence or absence of DHT by a quantitative real-time RT-PCR method. As shown in Figure 2A, DHT treatment showed 19–75% decrease in the levels of UGT1As in SVHUC-AR, compared to mock treatment. Next, we further studied the expression of UGT1A1, UGT1A4, and UGT1A9, because these subtypes have been proved to be important to detoxify bladder carcinogens, including aromatic amines and NNAL [10,11]. SVHUC-AR and SVHUC-V were treated with DHT and/or HF and mRNA expression of these three UGT1A subtypes were determined. As shown in Figure 2B–D, DHT treatment reduced the levels of UGT1A1, UGT1A4, and UGT1A9 by 31%, 31%, and 63%, respectively.
over mock treatment in SVHUC-AR. By contrast, DHT showed only marginal effects (<5% changes) in SVHUC-V. On UGT1A1 and UGT1A4, HF showed only marginal effects in SVHUC-AR and SVHUC-V, and it definitely blocked DHT effect in SVHUC-AR. On UGT1A9, HF itself showed 21% and 25% reduction in SVHUC-AR and SVHUC-V, respectively, and it partially antagonized DHT effect in SVHUC-AR. When compared with SVHUC-AR, SVHUC-V showed higher levels of these three UGT1A subtypes in any treatment group. In the presence of DHT, levels of UGT1A1, UGT1A4, and UGT1A9 in SVHUC-V were higher (2.1-, 4.6-, and 3.4-fold, respectively) than those in SVHUC-AR.

We also tested protein expression of UGT1A4 in SVHUC-AR and SVHUC-V treated with DHT and/or HF. As expected, similar changes in UGT levels were observed (Figure 2C, upper). These findings suggest that AR signals down-regulate UGT1As, even without androgens, in human normal urothelial cells.

Expression of Ugt1a in ARKO Mouse Bladder
To further investigate the involvement of AR signals in the regulation of Ugt1a expression in vivo, Ugt1a levels were analyzed in the bladders from ARKO mice. As shown in Figure 3, mRNA expression of all the Ugt1a subtypes was up-regulated in ARKO mice (1.4- to 3.5-fold) over wild-type male littermates. Although ARKO mice are known to have low levels of androgens [3,20], DHT supplement in these animals did not alter Ugt1a expression levels. These results further suggest that AR signals repress Ugt1a expression in mouse bladder and the effects of androgens on Ugt1a are mediated through the AR.

Expression of Ugt1a in Castrated Mouse Bladder
We then tested androgen effects on Ugt1a expression in male mouse bladder and also compared Ugt1a expression between male and female mice. Wild-type mice underwent bilateral orchiectomy or sham surgery followed by androgen or mock treatment. As shown in Figure 4, some Ugt1a subtypes, especially Ugt1a8, Ugt1a9, and Ugt1a10 (2.3-, 2.0-, and 1.6-fold, respectively), were up-regulated by castration in male mice. DHT supplement clearly eliminated the effect of castration on these three subtypes but induced marginal changes (<13%) in the levels of other subtypes. Of note were higher expression levels of all the subtypes of

Figure 2. Regulation of UGT1A expression by androgen and AR in SVHUC cells. (A) SVHUC-AR cultured in the presence of ethanol (mock) or 1 nM DHT for 48 h was analyzed on real-time RT-PCR for all the subtypes of UGT1A. SVHUC-AR and SVHUC-V cultured in the presence of ethanol (mock), 1 nM DHT, and/or 1 μM HF for 48 h were analyzed on real-time RT-PCR for UGT1A1 (B), UGT1A4 (C, lower), and UGT1A9 (D). Expression of each specific gene was normalized to that of GAPDH. Transcription amount is presented relative to that of mock treatment in SVHUC-AR [first lanes; set as 100% (A) or onefold (B–D)]. Each value represents the mean ± SD from at least three independent experiments. (C, upper) UGT1A4 protein expression. Equal amounts of protein extracted from each cell line were immunoblotted for UGT1A4 (56 kDa). β-Actin (43 kDa) served as the internal control. *P < 0.05 (vs. mock treatment in the same cell line). #P < 0.01 (vs. mock treatment in the same cell line).

Figure 3. Effects of ARKO on Ugt1a expression in mice. At 40 wk of age, urinary bladder from male wild-type mice (n = 3), ARKO mice (n = 3), and ARKO mice with DHT treatment (n = 3) were harvested and analyzed on real-time RT-PCR for all the subtypes of Ugt1a. Expression of each specific gene was normalized to that of GAPDH. Transcription amount is presented relative to that in the wild-type (first lanes; set as onefold). Each value represents the mean ± SE. *P < 0.05 (vs. wild-type male).
Ugt1a in female mice (1.3- to 2.1-fold over wild-type males).

Immunoreactivity of UGT1A in Normal Bladder and Bladder Cancer Tissue Samples

We performed immunohistochemical stains for UGT1A in 24 radical cystectomy specimens with high-grade urothelial carcinoma. Positive signals were detected predominantly in cytoplasm of epithelial cells (Figure 5A–D). The results of UGT1A expression in tissue samples are summarized in Table 2. Overall, all the non-neoplastic and neoplastic bladders showed at least weak signals in urothelial cells. Strong signals were found in 11 (46%) of 24 cancer tissues and in 17 (94%) of 18 corresponding benign tissues. Thus, the expression of UGT1A was significantly weaker in urothelial carcinoma than in benign urothelium (P = 0.0009). There were no statistically significant correlations between the intensity of UGT1A expression and gender, presence of muscle invasion (≤T1 vs. >T2), or lymph node metastasis. In the 24 bladder cancer specimens where AR expression had also been immunohistochemically analyzed [21], 9 of 16 (56%) AR-negative tumors showed strong UGT1A expression and 6 of 8 (75%) AR-positive tumors showed weak/moderate UGT1A expression. Thus, there was a tendency of inverse correlation between expressions of AR and UGT1A, but it was not statistically significant (P = 0.1557).

DISCUSSION

UGTs play a major role in the elimination of numerous carcinogens by: (i) transportation of the ultimate carcinogens excreted via the biliary or urinary tract; and (ii) sequestration of proximate carcinogens, leading to their detoxification [22]. The urinary bladder involves the main pathway for excretion of glucuronides and expresses all the subtypes of UGT except UGT2B17 [15]. Accumulating evidence has indicated that various transcription factors, such as hepatic nuclear factor-1α (HNF1α), HNF4α, arylhydrocarbon receptor (AhR),

Figure 4. Effects of castration on Ugt1a expression in mice. Wild-type male mice received sham surgery (n = 6), surgical castration (n = 6), or surgical castration followed by DHT supplement (n = 3) at 5 wk of age. Wild-type female mice received sham surgery (n = 6) at 5 wk of age. One week after the surgery, urinary bladders were harvested and analyzed on real-time RT-PCR for all the subtypes of Ugt1a. Expression of each specific gene was normalized to that of GAPDH. Transcription amount is presented relative to that in wild-type males with sham surgery (first lanes; set as onefold). Each value represents the mean ± SE. *P < 0.05 (vs. male control), #P < 0.01 (vs. male control).

Figure 5. Immunohistochemistry of UGT1A in benign and malignant bladder tissues. All benign bladder epithelia (A and B), except one case, showed a strong UGT1A immunoreactivity. In bladder carcinomas, strong (C) to weak (D) stains for UGT1A were observed. The wall of vessels was also positive for UGT1A, serving as a positive control. Original magnification, 200× (A, C, and D) or 400× (B). (E) Kaplan–Meier analysis of progression-free survival according to strong UGT1A expression. Comparison was made by log-rank test.

To assess possible associations between UGT1A staining and disease progression, we performed Kaplan–Meier analysis coupled with log-rank test. Of 24 patients with a mean follow-up of 10.1 months, 8 (33%) developed recurrent/metastatic tumors after radical surgery. As shown in Figure 5E, weak or moderate positivity of UGT1A was significantly associated with tumor progression (P = 0.0078).
and erythroid 2-related factor (Nrf2), have an impact on the activity of UGT genes [22]. In mouse urinary bladder, it was shown that BBN treatment reduced Ugt1a expression in a time and dose dependent manner and AhR signaling pathway was associated with this down-regulation [23,24]. It was also reported that knock-out of Nrf2 reduced Ugt1a expression and increased cancer incidence in BBN treated mice [23]. These data suggest that UGT in the urinary bladder functions to protect against chemical carcinogens. In the current study, we focused on UGT1A, especially UGT1A1, UGT1A4, and UGT1A9, that are known to be important in detoxifying bladder carcinogens, such as aromatic amines [10] and NNAL [11] in the liver. Nonetheless, because there is no clear functional correspondence between human UGT and mouse Ugt, the results on mouse model should be interpreted with caution.

Ligand activated nuclear receptors have been shown to regulate UGT expression [25]. Among them, AR was responsible for the gender difference in expression of some Ugt subtypes in mice [26] and rats [27]. Androgen itself is metabolized by some UGT subtypes and negative regulations of these enzymes by androgen have been reported [28]. In addition, a genome wide search identified UGT1A as a novel AR regulated gene in the prostate [18]. AR is also shown to suppress AhR activity by forming a complex [29]. Because AR, as a ligand-regulated transcription factor, likely promotes the development and progression of bladder cancer [3,30,31], these experimental observations formed the basis of our hypothesis: androgens regulate UGT1A expression in the bladder via the AR pathway, leading to male dominance in bladder cancer incidence. Although an AR-binding site (ARBS) was found in the non-promoter regions of UGT1As, it could influence the expression of only UGT1A1 and UGT1A3 whose transcriptional start sites are relatively close (>17 kb) to the ARBS [18]. We were unable to identify any putative ARBSs in each promoter region of UGT1A by a computer analysis, suggesting that ARBS(s) distant from the promoter region contribute to UGT1A regulation.

We first showed all the subtypes of UGT1A were down-regulated by androgen in the normal urothelial cell line SVHUC overexpressing the AR. The magnitudes of down-regulation are larger in UGT1A8, UGT1A9, and UGT1A10, compared to the others. It was likely that androgen functions through the AR pathway because an antiandrogen HF, at least partially, antagonized the effect of androgen in SVHUC-AR and no androgen effect was observed in SVHUC-V lacking a functional AR. In addition, HF down-regulated UGT1A9 in both SVHUC-AR and SVHUC-V cells. It has been reported that flutamide is glucuronidated by UGT [32] and is one of AhR activators [33]. Therefore, flutamide itself may regulate UGT1A9 via the pathway(s) other than AR. Of note were higher basal levels of UGT1As, particularly UGT1A4, in SVHUC-V than in SVHUC-AR and no androgen effect was observed in SVHUC-V lacking a functional AR. We then analyzed Ugt1a expression by using ARKO and castrated mouse models. As expected, all the Ugt1a subtypes were down-regulated by androgen in the normal urothelial cell line SVHUC overexpressing the AR. The magnitudes of down-regulation are larger in Ugt1a8, Ugt1a9, and Ugt1a10, compared to the others. It was likely that androgen functions through the AR pathway because an antiandrogen HF, at least partially, antagonized the effect of androgen in SVHUC-AR and no androgen effect was observed in SVHUC-V lacking a functional AR. In addition, HF down-regulated Ugt1a9 in both SVHUC-AR and SVHUC-V cells. It has been reported that flutamide is glucuronidated by UGT [32] and is one of AhR activators [33]. Therefore, flutamide itself may regulate UGT1A9 via the pathway(s) other than AR. Of note were higher basal levels of UGT1As, particularly UGT1A4, in SVHUC-V than in SVHUC-AR, suggesting down-regulation of UGT1As via non-androgen-mediated AR signals.

We then analyzed Ugt1a expression by using ARKO and castrated mouse models. As expected, all the Ugt1a subtypes were up-regulated in the bladders from ARKO mice with or without androgen supplement. However, castration resulted in increases in the expression of only Ugt1a8, Ugt1a9, and Ugt1a10, and androgen supplement eliminated this effect. These results, together with the data in cell lines, suggest that androgen is necessary for AR-mediated down-regulation of some UGT1A/Ugt1a subtypes, but AR signals induced by sub-physiological levels of androgens sufficiently...
down-regulate the other subtypes. Interestingly, female mice had higher expression of all the subtypes of Ugt1a. Since AR expression status in human bladder is similar between both sexes [34–36], ligand effects as well as those other than AR signals may contribute to gender difference in UGT expression. It has been reported that estradiol up-regulates some UGT subtypes in liver [37] and breast [38] cancer cell lines. Underlying mechanisms responsible for this gender difference need to be further explored.

In bladder cancer, the levels of UGT1A transcript were lower than those in normal counterpart [16]. An immunohistochemical study further revealed that UGT1A was strongly expressed in cytoplasm of normal bladder urothelium, whereas UGT1A expression was significantly decreased in urothelial carcinoma and was virtually negative in some high-grade tumors [17]. We confirmed UGT1A down-regulation in high-grade bladder cancer tissues, compared to non-neoplastic urothelium by immunohistochemistry. The present study also analyzed and compared the prognostic value of UGT expression by using Kaplan–Meier survival curves and log-rank test and expression status of UGT1A was found to significantly correlate with tumor progression. Interestingly, loss of strong UGT1A expression was observed in 47% of male patients versus 80% of female patients, although the difference was not statistically significant. The data may indeed support clinical evidence indicating women tend to present with less favorable tumor characteristics than men [39]. Overall, our immunostaining results suggest that UGT1A loss in bladder cancer could predict worse outcome.

We recently assessed AR expression, using the same bladder TMA, and found a strong correlation between AR positivity and tumor progression [21]. Expressions of AR and UGT, although there was no statistically significant difference, tended to be inversely correlated. These data may suggest that UGT1A down-regulated by AR signals also plays a preventive role in cancer progression. However, the sample size in our immunohistochemical study was relatively small, and, therefore, additional work with larger patient cohorts, including different grades of bladder tumor, should be done to conclude the relationship between AR and UGT expressions.

In conclusion, we showed down-regulation of UGT1A by androgen/AR in human normal bladder urothelial cells as well as up-regulation of Ugt1a in ARKO and castrated mouse bladders. Bladder cancer also exhibited lower levels of UGT1A expression, compared to normal urothelium. These results suggest that androgen-mediated AR signals play an important role in bladder cancer initiation by down-regulating the expression of UGT1As.

Further functional analyses of UGT in normal bladder/bladder cancer are necessary to determine their biological significance.

REFERENCES


