Androgen Glucuronidation: An Unexpected Target for Androgen Deprivation Therapy, with Prognosis and Diagnostic Implications

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Abstract

Androgen deprivation therapy (ADTh) remains a mainstay of prostate cancer treatment, but its efficacy is bypassed by mechanisms that are not fully understood. In human prostate cancer cells, androgen glucuronidation, catalyzed by the two UDP-glucuronosyltransferase (UGT) enzymes UGT2B15 and UGT2B17, is the major androgen inactivation pathway. In this study, we investigated the effect of ADTh on androgen glucuronidation to evaluate its potential clinical utility for prostate cancer prognosis or therapy. UGT2B15 and UGT2B17 expression was evaluated in prostate cancer specimens from untreated or treated patients and in cell models of prostate cancer exposed to clinically relevant antiandrogens. UGT2B15 and UGT2B17 protein levels in prostate were increased after 5 months of ADTh when compared with specimens from untreated patients. UGT2B15 expression remained elevated for up to 12 months, but UGT2B17 returned to initial levels as soon as after 6 months. Several androgen receptor (AR) antagonists tested caused a dose- and time-dependent stimulation of UGT2B15 and UGT2B17 expression and androgen glucuronidation in prostate cancer cell lines. The role of AR in these regulatory events was confirmed using AR-deficient LNCaP cells, in which UGT2B attenuation reduced the antiproliferative effects of AR pharmacologic antagonists. Through this combination of clinical and functional investigations, our work revealed that ADTh stimulates a local androgen metabolism in prostate cells, establishing a foundation to evaluate the potential of UGT2B15 and UGT2B17 as drug targets and/or molecular markers for ADTh responsiveness and maintenance in prostate cancer. Cancer Res; 73(23); 1–9. ©2013 AACR.

Introduction

Prostate cancer remains the second most frequent type of cancer diagnosed worldwide, and the third leading cause of cancer-related death in men in industrialized countries (1). Prostate cancer is an adenocarcinoma originating from cells of epithelial type, but displays morphologically and genetically very heterogeneous properties (2). Androgens are among the main factors controlling the initiation, maintenance, and progression of prostate cancer (3). During carcinogenesis, epithelial cells transform into a malignant phenotype, where the androgen receptor (AR) selectively activates genes positively controlling cell viability to unrestraint epithelial cell proliferation (3). For this reason, androgen deprivation therapy (ADTh), also called ‘medical castration’ or ‘hormone therapy,’ has become a first-line treatment strategy for advanced prostate cancer (4). ADTh is achieved using gonadotropin releasing hormone (Gn-RH) analogs such as leuproide, buserelin, or goserelin (4), which inhibit testosterone synthesis (2). This medical castration is often combined to antiandrogens such as flutamide, nilutamide, or bicalutamide (2). These AR antagonists provide an additional blockade of androgen signaling by preventing AR activation by the locally synthesized androgens. Symptomatic (improvement of quality of life) and/or objective (serum prostate-specific antigen level normalization and measurable tumor response) outcomes to ADTh are observed in approximately 80% of patients (5). However, the duration of these improvements is highly variable, and prostate cancer relapses in the majority of patients evolving to the androgen-independent phenotype within 12 to 18 months of therapy (5). The cancer is then commonly referred to as castrate-resistant prostate cancer (CRPC), androgen independent, or hormone refractory (6). CRPC can transiently be treated with alternative ADTh, but the ultimate options for androgen-independent metastatic prostate cancer lie in chemotherapy (5, 7).

The androgen axis continues to play a major role for the progression of CRPCs (8), and a deep understanding of factors affecting androgen inactivation in tumor cells is required. In

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the prostate, the active hormone dihydrotestosterone (DHT) is extensively metabolized to inactive and easily excretable androstane-3α-diol glucuronide (3α-diol-17G) and androstone-17α-glucuronide (ADT-3G) derivatives (9, 10). These polar metabolites are formed through a conjugation reaction called glucuronidation, and correspond to the major androgen metabolic end products found in circulation in men (11, 12). In humans, glucuronidation is catalyzed by the 19 functional UDP-glucuronosyltransferase (UGT) enzymes (13). However, in prostate, androgen glucuronidation only involves two enzymes: UGT2B15 and UGT2B17 (14). Experimental inhibition of these enzymes significantly improves the propropriaferative properties of DHT in prostate cancer cells (14), suggesting that they are major determinants for the androgen response. Accordingly, positive associations were reported between prostate cancer risk and a low-activity UGT2B15 allele or a complete UGT2B17 gene deletion (15–19). On the other hand, various studies illustrated the negative control that androgens exert on UGT2B15 and UGT2B17 expression in prostate cancer cells (14, 20, 21). These last observations establish the AR regulatory pathway as a major mechanism for controlling androgen glucuronidation. We therefore sought to test the possibility that ADTh drugs affect UGT2B15 and/or UGT2B17 expression or activity in prostate cancer tumor samples and cell models exposed to antiandrogens.

Materials and Methods

Materials

UDP-glucuronic acid and all aglycons were obtained from Sigma or ICN-Pharmaceuticals Inc. R1881, 3α-Diol, DHT, ADT, and DHT-glucuronide (DHT-G) were purchased from Steraloids. ADT-3-glucuronide and 3α-Diol-17-glucuronide were provided by the Medicinal Chemistry Division of the "Centre de recherches du CHU-Québec" (21). Cell culture media, plastic, and lipofectin, were purchased from Invitrogen. Penicillin, streptomycin, and Iscove’s Modified Dulbecco’s Medium (IMDM) were provided by Wisent, whereas puromycin, doxycyclin, bicalutamide, nilutamide, flutamide, and RPMI were purchased from Sigma. SYBR Green PCR Master Mix was purchased from Applied Biosystem (Life Technologies). Protein assay reagents were obtained from Bio-Rad Laboratories Inc. The anti-calnexin antibody was purchased from Stressgen, the anti-vimentin antibody was from GenScript, and the anti-UGT2B15 and anti-UGT2B17 antibodies were described previously (22, 23). The secondary antibody against rabbit immunoglobulin G (IgG) was purchased from Amersham. The chemiluminescence kit (ECL) was from Renaissance. SiRNA probe for UGT2B15/UGT2B17 (Individual siGENOME duplex D-020195-01) and Non-Target #1 were obtained from Dharmacon as already described in ref. 14.

Tissue banks

Tissues used for the microarray construction were from the Vancouver Prostate Centre Tissue Bank (http://www.prostatecentre.com/our-research/core-facilities/biorepository), as described in refs. 24, 25. Prostate samples were obtained from 31 benign prostatic hyperplasia (BPH) donors, whereas prostate cancer samples were from 88 patients with advanced prostatic hyperplasia: 16 from untreated donors, 16 from participants with CRPC, and finally 56 from patients having received ADTh for 1 to 5 months ($n = 19$), 6 to 8 months ($n = 24$), or 9 to 12 months ($n = 13$; Supplementary SM1). Tissue samples were extracted from the whole tissue after radical prostatectomy, except CRPC samples that were obtained by transurethral resection of the prostate (24, 25). The therapy consisted of Gn-RH analogs (buserelin, leuprolide, or goserelin) and antiandrogens (flutamide, cyproterone acetate, or bicalutamide) used alone or in combination. This protocol was approved by the institutional review boards at the University of British Columbia (Vancouver, BC, Canada), and the "Centre hospitalier universitaire de Québec" (Québec, QC, Canada). Written informed consent was obtained from all participants.

Tissue microarrays

Microarrays and immunohistochemical (IHC) stains were obtained as previously described (24, 25), with formalin-fixed and paraffin-embedded 4-μm sections of the microarray, using the previously described (22, 23, 26) polyclonal anti-vimentin (positive control; 1:100 dilution), anti-UGT2B15 (1:300 dilution), and anti-UGT2B17 (1:150 dilution) antibodies (Supplementary SM2–8). The IHC staining (Supplementary SM2–8) was scored by a pathologist (L. Fazli) for the level of immunoeexpression on a scale from 0 to 3, wherein 0 was undetectable, 1 represented a faint or focal questionably present stain, 2 represented a stain of convincing intensity in a minority of cells, and 3 represented a stain of convincing intensity in a majority of cells.

Cell culture

Cell lines were obtained from the American Type Culture Collection (cell authentication through short tandem repeat profiling), used within 6 months following reception. LNCaP cells were grown and treated in 10% FBS-supplemented RPMI 1640 medium. LAPC-4 cells were cultured in IMDM completed with 7.5% FBS and 10 nmol/L R1881. Inducible AR-shRNA-expressing LNCaP cells were cultured in RPMI 1640 supplemented with 10% FBS, puromycin (2.5 μg/mL), and blasticidin (1 μg/mL), as reported in ref. 27. AR deficiency was obtained through a 48-hour pretreatment with doxycycline (DOX, 1 μg/mL), and the antibiotic was maintained for the complete treatment duration as recommended (27). For RNA isolation, 2.5 × 10^5 LNCaP or 3.5 × 10^5 LAPC-4 cells were plated in each well of 12-well plates. For glucuronidation assays and Western blot analysis experiments, 8 × 10^5 LNCaP cells were plated in 10-cm petri dishes. Cells were then treated for the indicated duration with vehicle [dimethyl sulfoxide (DMSO)] or ethanol; 0.1% v/v), bicalutamide (DMSO), nilutamide (ethanol), flutamide (ethanol), R1881 (ethanol), and/or DHT (ethanol) at the indicated concentrations. Knockdown of UGT2B15 and UGT2B17 expression was obtained using a UGT2B15/UGT2B17 siRNA probe as already reported in ref. 14. Following transfection, cells were allowed 18 hours for recovery, transferred in 96-wells plates, and exposed to vehicle (DMSO; 0.1% v/v) or bicalutamide (10 μmol/L) for 72 hours. Cell proliferation was then ensured using the CellTiter 96 AQueous One Solution Cell Proliferation Assay Kit (Promega) and
Androgen ablation therapy modulates UGT2B15 and UGT2B17 protein levels in prostate tumors. UGT2B15 (A) and UGT2B17 (B) protein levels were quantified in prostate tumors using IHC analyses of a tissue microarray with specific antibodies as illustrated in Supplementary SM2–8. Tumor samples were from 31 BPH donors or 88 patients with advanced prostate cancer (Supplementary SM9), who were either untreated (n = 16) or treated with ADTh for 1 to 5 months (1–5; n = 19), 6 to 8 months (6–8; n = 24), and 9 to 12 months (9–12; n = 13), or with castrate-resistant tumors (CRPC; n = 16). The staining intensity was visually scored on a scale of 0 to 3 (see Materials and Methods), and values represent the mean ± SEM of the scores obtained for each experimental group (expressed relatively to untreated samples). Statistically significant differences between experimental groups are indicated by asterisks when compared with untreated and by ¥ when compared with BPH (Mann–Whitney test: *, P < 0.05; **, P < 0.01; † † †, P < 0.001; ¥, P < 0.05; NS, not significant).

Genotyping of the UGT2B17 gene copy number variation
Genomic DNA was isolated from LNCaP and LAPC-4 cells by a phenol protocol as specified by the supplier (Promega). Genotyping of the UGT2B17 gene copy number variation was then used as a template for quantification using the ChargeSwitch gDNA Mini Tissue Kit (Life Technologies) and was then used as a template for UGT2B17 deletion genotyping as previously described (25, 28).

RNA isolation and quantitative reverse transcription PCR
Total RNA was isolated according to the Tri-Reagent acid/phenol protocol as specified by the supplier (Molecular Research Center Inc.). The reverse transcription (RT) and quantitative PCR reactions were performed as previously described (25).

Western blot analyses and in vitro glucuronidation
Western blot analyses and in vitro glucuronidation assays were performed as previously reported (14, 21, 22).

Statistical analyses
All data are presented as mean ± SD, except for the IHC intensity evaluation in which results are presented as mean ± SEM. Comparisons between experimental groups were performed by a two-tailed Student t test or a Mann–Whitney U test for in vivo assessment of UGT levels using the JMP V7.01 software (SAS Institute).

Results
Androgen ablation therapy modulates UGT2B15 and UGT2B17 protein levels in prostate tumors
According to our previous observation (25), untreated prostate cancer samples presented similar UGT2B15 and UGT2B17 protein levels as in BPH samples (Fig. 1; Supplementary SM2–8). However, when compared with BPH and untreated samples, both UGT proteins were significantly more abundant in tissues from ADTh-treated patients for 1 to 5 months (Fig. 1; Supplementary SM2–5). The highest UGT2B15 expression was observed after 6 to 8 months of treatment, and remained significantly higher than in untreated samples up to 12-month therapy, even if a tendency to lower again was observed in these last samples (Fig. 1A). In contrast, UGT2B17 protein levels were reduced to basal levels (untreated) as soon as after a 6- to 8-month period of treatment and remained stable for longer exposures (Fig. 1B). In patients with CRPCs, both UGT2B15 and UGT2B17 protein levels were not significantly different from those in untreated samples. In the IHC results obtained with positive control, vimentin (29) indicated that the quantification of UGT2B protein levels was not biased by the tissue preparation (Supplementary SM2–8).

Androgen receptor antagonism upregulates UGT2B15 and UGT2B17 expression and activity in prostate cancer cell models
Ex vivo experiments were performed using prostate cancer cell models and AR antagonists to further investigate the effects and consequences of AR blockade on androgen glucuronidation (Figs. 2–4).

First, UGT2B15 and UGT2B17 mRNA and protein levels were quantified in LNCaP cells exposed to increasing doses of bicalutamide (0.1 to 20 μmol/L) for 48 hours. The two UGTs were dose-dependently increased at both mRNA and protein levels (Fig. 2). Although the amount of UGT2B15 transcripts was statistically increased in the presence of as low as 1 μmol/L (Fig. 2A), UGT2B17 mRNA accumulation reached the statistical significance only in the presence of 5 μmol/L bicalutamide (Fig. 2B). In time course experiments, UGT2B15 and UGT2B17 optical density determination (490 nm) was done with a microplate reader Tecan Infinite M1000 as recommended by the supplier (Promega).

Figure 1. Androgen ablation therapy modulates UGT2B15 and UGT2B17 protein levels in prostate tumors. UGT2B15 (A) and UGT2B17 (B) protein levels were quantified in prostate tumors using IHC analyses of a tissue microarray with specific antibodies as illustrated in Supplementary SM2–8. Tumor samples were from 31 BPH donors or 88 patients with advanced prostate cancer (Supplementary SM9), who were either untreated (n = 16) or treated with ADTh for 1 to 5 months (1–5; n = 19), 6 to 8 months (6–8; n = 24), and 9 to 12 months (9–12; n = 13), or with castrate-resistant tumors (CRPC; n = 16). The staining intensity was visually scored on a scale of 0 to 3 (see Materials and Methods), and values represent the mean ± SEM of the scores obtained for each experimental group (expressed relatively to untreated samples). Statistically significant differences between experimental groups are indicated by asterisks when compared with untreated and by ¥ when compared with BPH (Mann–Whitney test: *, P < 0.05; **, P < 0.01; † † †, P < 0.001; ¥, P < 0.05; NS, not significant).
transcripts were significantly increased as soon as after 12 hours of treatment, and the maximal induction was reached after a 48-hour exposure (Fig. 2C and D). A similar dose- and time-dependent accumulation was observed for both UGT proteins in Western blot analyses (Fig. 2).

In vitro glucuronidation assays confirmed that the bicalutamide-dependent increase in UGT expression results in improved androgen conjugation (Fig. 3). Indeed, LNCaP cells exposed to bicalutamide exhibited a dose- and time-dependent improved ability to conjugate DHT (Fig. 3A and D), ADT (Fig. 3B and E), and 3α-diol (Fig. 3C and F).

To ascertain that results from bicalutamide-treated LNCaP cells were not cell type- or antagonist specific, additional experiments were performed using the LAPC-4 cell model and the other AR antagonists, nilutamide and flutamide (Fig. 4). In LAPC-4, bicalutamide dose- and time-dependently induced UGT2B15 expression (Fig. 4A and B). UGT2B17 transcripts were not detected in these cells. Actually, genotyping analyses revealed that the UGT2B17 gene is absent in LAPC-4 (data not shown), indicating their del/del phenotype for the previously reported UGT2B17 CNV genotype (28). In both LNCaP and LAPC-4 cells, bicalutamide and flutamide were efficient in increasing UGT2B15 mRNA levels (Fig. 4C and D). A similar response was also observed for UGT2B17 transcripts in LNCaP cells (Fig. 4C). Interestingly, nilutamide exhibited a cell-specific response with a significant induction of UGT2B15 mRNA in LAPC-4 but not in LNCaP cells (Fig. 4C and D). Furthermore, not only did nilutamide not improve UGT2B17 expression, but it caused a non-significant reduction of these transcripts in LNCaP cells (Fig. 4C). This last observation is consistent with the previously reported agonistic effects of nilutamide in front of the mutated AR that is expressed in LNCaP cells (30).

The androgen receptor mediates the bicalutamide-dependent upregulation of UGT2B15 and UGT2B17 expression in LNCaP cells

AR contribution to the bicalutamide-dependent induction of UGT2B15 and UGT2B17 expression was ensured by using LNCaP cells expressing a DOX-inducible anti-AR short hairpin RNA (shRNA; Fig. 5; ref. 27). We first confirmed that DOX treatment resulted in impaired AR expression and activity (Supplementary SM9; ref. 27). Subsequently, native (DOX−) and DOX-activated cells (DOX+) were cultured in the presence of vehicle, bicalutamide (10 μmol/L), or the synthetic (R1881; 1 nmol/L), or natural (DHT, 10 nmol/L) AR activators (Fig. 5).

In native (DOX−) cells, DHT, R1881, and bicalutamide caused the expected down- (R1881 and DHT; ref. 21) or
upregulation (bicalutamide) of UGT2B15 (Fig. 5A) and UGT2B17 (Fig. 5B) mRNA expression.

In AR-deficient (DOX−) cells, DOX alone also significantly increased the UGT2B15 (Fig. 5A) and UGT2B17 (Fig. 5B) transcript levels, thus revealing that inhibition of AR expression has similar consequences as the use of AR antagonists for these UGT gene expressions. The addition of R1881 in culture media had no further impact on mRNA levels of the

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two UGTs (Fig. 5). Similarly, the negative effect of DHT on UGT2B15 expression was completely lost, as its mRNA levels in vehicle- and DHT-treated DOX\(^{-}\) cells were not significantly different (Fig. 5A). In contrast, UGT2B17 mRNA levels were found significantly more abundant in DHT-treated than in vehicle DOX\(^{-}\) cells (Fig. 5B). Also surprising was the observation that bicalutamide reduces UGT2B15 and UGT2B17 mRNA levels in cells cultured with DOX (Fig. 5). This last observation suggests that bicalutamide exerts opposite effects on UGT genes’ expression in the presence of high (DOX\(^{-}\)) or low (DOX\(^{+}\)) AR levels. However, when mRNA levels were compared with those quantified in bicalutamide-treated native cells, only the strong reduction in UGT2B17 transcripts remained statistically significant (Fig. 5B), indicating that the loss of AR mainly altered the response of this enzyme to bicalutamide.

Nonetheless, the fact that AR knockdown abolishes or reverses the effects of bicalutamide confirms the role played by this receptor in the antiandrogen-induced upregulation of UGT2B15 and UGT2B17 expression.

**Knockdown of UGT2B15 and UGT2B17 reduces the antiproliferative properties of bicalutamide**

We next evaluated whether induction of UGT2B15 and UGT2B17 expression participates in the antiproliferative properties of bicalutamide (31). For this purpose, LNCaP cells were transfected with a nontarget (siRNA control) or the anti-UGT2B15/UGT2B17 siRNA probe (Fig. 6A; ref. 14), and then exposed to vehicle (DMSO) or bicalutamide (10 \(\mu\)mol/L) for up to 4 days (Fig. 6B). As expected (31), bicalutamide caused a significant 24.5% reduction of control cells’ proliferation after 3 days of exposure. This reduction was further enhanced to 32.5% inhibition after 4 days (Fig. 6B). Interestingly, UGT2B-deficient cells were less sensitive to the antagonist and their proliferation was only 15% and 22.5% reduced in the presence of the drug after 3 and 4 days, respectively (Fig. 6B). Thus, siRNA-mediated knockdown of UGT2B15 and UGT2B17 expression provoked 40% and 32% reductions of the antiproliferative effects of bicalutamide in LNCaP cells exposed to the drug for 3 and 4 days, respectively.

**Discussion**

This study evidences a novel and incidental effect by which androgen ablation drugs abolish the ability of carcinogenic androgens to inhibit their own inactivation in prostate cancer cells. This first comprehensive analysis of AR blockade consequences for androgen glucuronidation identifies the two androgen-conjugating UGT2B15 and UGT2B17 enzymes as ADTh-positive targets, and establishes the major contribution of these effects to the antiproliferative properties of ADTh drugs.

An interesting observation of the present study is the differential manner in which the 2 UGT genes respond to AR blockade in and ex vivo. These differences are thought to reflect variations in the AR-dependent modulation of the two UGT genes. Indeed, the fact that AR agonists/antagonists fail to modulate UGT2B15 expression in receptor-deficient cells, while having inverse effects on UGT2B17 mRNA levels, suggests that alternative regulatory processes drastically modify the AR-dependent modulation of UGT2B17 expression when the androgen receptor is reduced. Although thorough investigations are required to fully grasp the mechanisms governing the molecular switch in the UGT2B17 response, it can be envisioned that such UGT-specific processes may be derived from nucleic acid differences in the transcriptional regulatory
regions of the UGT2B15 and UGT2B17 genes. Even if the two genes share considerable sequence homology (9), previous studies already illustrated that minor differences in their proximal promoters are responsible for their differential response to various regulatory pathways (32, 23). Such changes actually exist within the AR response elements identified in UGT2B15 and UGT2B17 promoter sequences (20), and thus may play a role in their isoform-specific response under low AR levels. Other regulatory pathways may also be involved. For example, EGF signaling, which downregulates UGT2B17 expression without affecting UGT2B15 in LNCaP cells (23), has been identified as a transduction pathway involved in prostate tumor growth (33, 34).

Beyond these mechanistic considerations, the present results are of clinical significance for prostate cancer progression, treatment, and diagnosis. In terms of tumor progression, the desensitization of the UGT response to ADTh, as revealed by the similar protein levels detected in untreated tumors and CRPCs, suggests that the loss of UGT induction is involved in tumors’ transition to castration resistance. Even if resistant to ablation therapies, CRPC progression remains dependent on the androgen axis (8, 35). Because AR acts as a negative UGT2B15 and UGT2B17 gene–regulator (14, 20, 21), the loss of UGT induction detected in vivo may actually reflect an AR reactivation in resistant tumors. On the other hand, as observed with UGT knockdown experiments, the induction of androgen glucuronidation may contribute to the antiproliferative effects of AR antagonists. It is therefore tempting to speculate that the loss of this induction is likely involved in the phenotypic changes allowing tumor cells to proliferate in the presence of low androgen levels, as observed in CRPCs (8). This idea is supported by the inverse relationship existing between UGT2B15 and UGT2B17 expression and androgen-dependent prostate cancer cell proliferation (14) and by the strong influence that UGT2B15 and UGT2B17 polymorphisms exert on tissue androgen levels (36). The loss of UGT genes’ sensitivity may allow prostate cancer cells to maintain sufficient DHT levels to activate AR and its regulated genes in CRPCs (37, 38). On the basis of these observations, one can conclude that the UGT induction in short-term treated samples is an important biochemical component of the initial ADTh benefits (39). Following on that point, it can be envisioned that UGT2B15 and UGT2B17 are actually underexploited therapeutic targets for androgen deprivation therapies. Until now, antiandrogenic approaches have been focusing on androgen biosynthesis and activity (39). The present study validates the potential of targeting glucuronidation to reduce active androgen concentrations in prostate cancer cells, thus providing a strong rational for searching pharmacologic agents that stimulate androgen glucuronidation in prostate cancer cells. However, such an alternative strategy requires the identification of AR-independent UGT2B15 and UGT2B17 gene–inducers.

The present work may also have a significant impact in terms of prostate cancer prognosis and diagnosis. Indeed, the respective changes in UGT2B15 and UGT2B17 protein levels in response to ADTh identify these two proteins as potential biomarkers both for evaluating the responsiveness to antiandrogen drug therapies and preventing resistance occurrence. ADTh remains the cornerstone of systemic treatments for locally advanced prostate cancer (38), and biochemical predictors are currently needed to assess whether additional treatments should be initiated (40). Furthermore, patients with CRPC have a poor prognosis and account for the majority of prostate cancer–related deaths (39), thus being able to anticipate that ADTh resistance can be helpful in adapting treatment settings with new effective antiandrogenic agents to prevent the development of castrate-resistant tumors (39). A continuous follow-up of

Figure 6. UGT2B15- and UGT2B17-deficient LNCaP cells are less sensitive to the antiproliferative effects of bicalutamide. A and B, LNCaP cells were transiently transfected with a nontarget (control) or an anti-UGT2B15/UGT2B17 siRNA probe (UGT deficient) using lipofectin for 6 hours, allowed 18 hours for recovery, and then exposed to vehicle (DMSO) or bicalutamide (10 μmol/L) for up to 2, 3, or 4 days (D). A, UGT2B15 and UGT2B17 mRNA levels were quantified from total RNA through quantitative RT-PCR analyses and normalized with the housekeeping RNA 36B4. Values (mean ± SD) are expressed as number of cells per well. Percentages of reduction in cell proliferation were determined. Statistically significant differences between siRNA control and siRNA UGT2B15/B17-transfected cells are indicated by asterisks (Student t test: *, P < 0.05; **, P < 0.01; ***, P < 0.001; NS, not significant).
UGT2B15 and/or UGT2B17 intraprostatic protein levels from the time of diagnosis constitutes a potential approach to evaluate therapy responsiveness, and measuring UGT2B17 levels may also be helpful to anticipate the loss of optimal ADT effects in UGT2B17-positive patients, and then initiate alternative therapeutics, even before the resistance occurrence.

The earlier-discussed diagnostic and therapeutic opportunities are mainly based on results from the tissue microarray study, while largely supported by results from functional investigations. However, sample heterogeneity is a major problem in tissue array studies, especially in prostate cancer, in which patients receive varied drugs or combination of drugs, and the tumor areas are small and often surrounded by normal cells (40). Therefore, the role of UGT2B15 and UGT2B17 as drug targets and/or molecular biomarkers remains to be validated through large and accurate clinical settings.

Nevertheless, the combination of clinical and functional approaches used for the current investigation demonstrates that ADT drugs stimulate the local androgen metabolism in prostate cells, and identifies UGT2B15 and UGT2B17 as potential anticancer drug targets and/or biomarkers for androgen ablation responsiveness and maintenance.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors' Contributions
Conception and design: L. Grosse, O. Barbier
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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): L. Grosse, S. Paquet, P. Caron, P.S. Rennie
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): L. Grosse, S. Paquet, A. Bélanger, O. Barbier
Writing, review, and/or revision of the manuscript: L. Grosse, S. Paquet, O. Barbier

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