UDP-glucuronosyltransferase 2B15 (UGT2B15) and UGT2B17 Enzymes Are Major Determinants of the Androgen Response in Prostate Cancer LNCaP Cells*

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Uridine diphosphate-glucuronosyltransferase 2 (UGT2)B15 and B17 enzymes conjugate dihydrotestosterone (DHT) and its metabolites androstane-3α,17β-diol (3α-DIOL) and androsterone (ADT). The presence of UGT2B15/B17 in the epithelial cells of the human prostate has been clearly demonstrated, and significant 3α-DIOL glucuronide and ADT-glucuronide concentrations have been detected in this tissue. The human androgen-dependent cancer cell line, LNCaP, expresses UGT2B15 and -B17 and is also capable of conjugating androgens. To assess the impact of these two genes in the inactivation of androgens in LNCaP cells, their expression was inhibited using RNA interference. The efficient inhibitory effects of a UGT2B15/B17 small interfering RNA (siRNA) probe was established by the 70% reduction of these UGT mRNA levels, which was further confirmed at the protein levels. The glucuronidation of dihydrotestosterone (DHT), 3α-DIOL, and ADT by LNCaP cell homogenates was reduced by more than 75% in UGT2B15/B17 siRNA-transfected LNCaP cells when compared with cells transfected with a non-target probe. In UGT2B15/B17-deficient LNCaP cells, we observe a stronger response to DHT than in control cells, as determined by cell proliferation and expression of eight known androgen-sensitive genes. As expected, the amounts of DHT in cell culture media from control cells were significantly lower than that from UGT2B15/B17 siRNA-treated cells, which was caused by a higher conversion to its corresponding glucuronide derivative. Taken together these data support the idea that UGT2B15 and -B17 are critical enzymes for the local inactivation of androgens and that glucuronidation is a major determinant of androgen action in prostate cells.

Production and secretion of testosterone by the testis has long been considered one of the major factors influencing androgen function in androgen target tissues, namely, the prostate. Thus, circulating testosterone taken up from the circula-

The glucuronidation of dihydrotestosterone (DHT), the natural androgen receptor agonist (1), is converted by 5α-reductase to dihydrotestosterone (DHT), the natural androgen receptor agonist (1). There is clear evidence now that adrenal steroid precursors, namely dehydroepiandrosterone and its sulfate, are converted to testosterone in several tissues (2, 3). The observation that the DHT concentration in prostate is only decreased by 50% in castrated patients treated for prostate cancer further support the role of adrenal steroid precursors as a source of androgens (4–6). These findings of local transformation of circulating steroids into bioactive testosterone and DHT have been integrated in the process called “intracrinology,” where several steroidogenic enzymes, including 3α-hydroxysteroid dehydrogenase-Δ4–5 isomerase (3α-HSD type 1), 17β-HSDs, and 5α-reductase, contribute to the formation of DHT (2, 7). Growing evidence indicates that tissue DHT concentrations are also modulated by 3α-hydroxysteroid dehydrogenase (3α-HSD) type 3 and 17β-HSD type 7, which form inactive androstan-3α,17β-diol (3α-DIOL) and androsterone (ADT) (8, 9). Although extremely important in modulating tissue DHT levels, these steroidogenic enzymes are reversible. By contrast, glucuronidation of these substrates, namely DHT, 3α-DIOL, and ADT, is an irreversible process of steroid metabolism, and this conjugation is present in several androgen target tissues (10–13).

Uridine diphosphate-glucuronosyltransferase (UGT) enzymes inactivate substrates by the addition of the glucuronyl moiety of the UDP-glucuronic acid (UDP-GA) (14, 15). Thus, the bulky polar metabolite is no longer able to interact with its corresponding receptor, and its excretion is facilitated by the increase in water solubility (15, 16). Of the 17 UGT enzymes identified in humans, only 3 conjugate testosterone and its metabolites at physiologically relevant levels: UGT2B7, -B15, and -B17 (2). UGT2B7 and -B17 conjugate ADT at the position 3-hydroxylation, whereas only UGT2B7 forms 3α-DIOL-3-glucuronide. UGT2B15 and -B17 specifically conjugate DHT and 3α-DIOL at the 17-hydroxyl position (2, 17). 3α-DIOL-17G is the predominant form of circulating 3α-DIOL-glucuronide in adult men and has been identified as a strong predictor of prostate volume (18). This was in agreement with previous reports indicating the presence of UGT2B15 and -B17 tran-

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The abbreviations used are: DHT, dihydrotestosterone; DHT-G, DHT-glucuronide; 3α-DIOL, androstan-3α,17β-diol; ADT, androsterone; UGT2B15-glucuronosyltransferase 2; UDPGA, UDP-glucuronic acid; LC, liquid chromatography; MS, mass spectometry; RT, reverse transcription; VEGF, vascular endothelial growth factor; AR, androgen receptor; siRNA, small interfering RNA; PSA, prostate-specific antigen.
scripts in the prostate and the localization of both enzymes by immunohistochemistry in luminal and basal epithelial cells, respectively (19, 20).

Although circulating testosterone and adrenal steroid precursors and several local steroidogenic enzymes are implicated to ensure production of tissue DHT in a given tissue and, consequently, the expression of androgen-dependent genes, it is now clear that androgen-conjugating enzymes also contribute to local androgen homeostasis. Most interestingly, the presence of a single nucleotide polymorphism within the coding region of the UGT2B15 gene, which results in an amino acid change from an aspartic acid to a tyrosine at position 85, has been previously reported (11). The UGT2B15D85 enzyme is less efficient in glucuronidating DHT, and it was postulated that the presence of this low activity allele would result in higher intra-prostatic DHT concentrations (11, 19). Recent genotyping studies (21–24) revealed that the homozygous UGT2B15D85 allele was significantly more common in prostate cancer patients than in control individuals, thus supporting the concept that alteration in androgen inactivation could be implicated in androgen function and prostate cancer. In the present study we have tested the hypothesis that glucuronidation of androgen in LNCaP cells may alter the expression of androgen-dependent genes.

In androgen-dependent prostate cancer cells, LNCaP, UGT2B15 and -B17 are highly expressed (17) and the conjugation of androgen metabolites such as DHT, 3α-DIOL, and ADT have been demonstrated (2, 25). The presence of UGT2B15/B17 in LNCaP cells was particularly useful to investigate the regulation of their expression by several factors, including androgen, growth factors, and cytokines (17, 26–28).

In the present study we have investigated the effect of knocking down UGT2B15 and -B17 expression on the formation of glucuronides and on the response of androgen-sensitive genes to DHT treatment in LNCaP cells. Our data demonstrate for the first time that changes in UGT2B activity alter the androgen-dependent gene expression.

**EXPERIMENTAL PROCEDURES**

**Materials**—R1881 was purchased from PerkinElmer Life Sciences. Protein assay reagents were obtained from Bio-Rad. UDPGA and [14C]UDPGA were obtained from Sigma and PerkinElmer Life Sciences, respectively. 3α-DIOL, DHT, and DHT-glucuronide (DHT-G) were purchased from Steraloids (Newport, RI). 3α-DIOL-3-glucuronide, 3α-DIOL-17-glucuronide, and Casodex were provided by the Medicinal Chemistry Division of our laboratory (17). Ammonium formate was from Aldrich, and high performance liquid chromatography-grade methanol was provided by VWR Canlab (Montréal, Québec, Canada). Human prostate cancer (LNCaP) cells were obtained from the American Type Culture Collection (Manassas, VA). siRNA for UGT2B15/B17 (Individual siGENOME duplex D-020195-01) and Non-Target #1 were obtained from Dharmacon (Chicago, IL). SYBRGreen PCR Mix 2X was obtained from Applied Biosystems (Foster City, CA). The CellTiter 96 Aqueous One Solution Cell Proliferation Assay was purchased from Promega (Fisher Scientific, Ltd., Nepean, Ontario, Canada).

**Cell Culture**—LNCaP cells were grown as previously described (25, 27). For transfections with siRNA, LNCaP cells were plated at 2.5 × 10⁴, 5 × 10⁴, 2 × 10⁵, and 5 × 10⁵ cells/well in 96, 24, 12, and 6 wells plates, respectively. Twenty-four hours before transfection, medium was changed for RPMI without serum, and transfections were performed for 24 h using 2 μl of Lipofectin per μg of siRNA in Opti-MEM (Invitrogen). siRNA concentrations ranged from 25 to 100 nm in dose-response experiments (see Table 2), and for time-course experiments, cells were cultured for 24–96 h post-transfections in RPMI with 2% charcoal stripped fetal bovine serum. For glucuronidation assays, cells were homogenized 72 and 96 h after transfections. When required, DHT, R1881, or Casodex was added to media 96 h post-transfection for a duration of 12 h (gene expression assays and media DHT and DHT-G assays) or 72 h post-transfection for a duration of 48 h (cell proliferation assays). 20 μl of Cell Proliferation Quantification kit (Promega) was added to each well for 2 h, and absorbance was quantified at 490 nm. AR agonists were dissolved in ethanol, and the volume added to media never exceeded 0.1% ethanol (v/v). For longer treatments, fresh medium was added every 48 h.

**Western Blot Experiments**—LNCaP cell homogenates were prepared in phosphate-buffered saline solution with dithiothreitol (0.5 mm). For Western blot experiments, 10–20 μg of total proteins were separated by 10% SDS-polyacrylamide gel. Gels were transferred onto nitrocellulose membranes and probed with anti-UGT2B15 (1849 at 1:1500 dilution) (20), anti-UGT2B17 (EL-95 at 1:2000 dilution) (11), or the anti-calnexin antibodies (1:5000 dilution) as internal control (Stressgen, Victoria, British Columbia, Canada). An anti-rabbit IgG horse antibody conjugated with peroxidase (Amer sham Biosciences) was used as the secondary antibody, and the resulting immunocomplexes were visualized using a chemiluminescence kit (ECL) (Renaissance, Quebec, Quebec, Canada) and exposed on Hyperfilm® (Eastman Kodak Co) for 15–60 s.

**Glucuronidation Assay and Steroid Assays**—Enzymatic assays were performed using 10–20 μg of total proteins in 50 mM Tris-buffered saline (pH 7.4) with dithiothreitol (0.5 mm) in the presence of 1 mM UDPGA, 10 mM MgCl₂, 50 mM Tris-HCl (pH 7.5), 100 μg/ml phosphatidylcholine, 8.5 mM saccharolactone, and 200 μM concentrations of the different substrates (25). Assays were incubated at 37 °C for 1 h and terminated by adding 100 μl of methanol followed by centrifugation at 14,000 × g for 10 min, as previously described (13). The formation of glucuronide conjugates in cell homogenate and in cell media DHT-G was measured by using liquid chromatography coupled with mass spectrometry (LC-MS/MS) as previously described (25). Non-conjugated DHT was determined by gas chromatography mass spectrometry as previously described (29). The formation of DHT- and R1881-glucuronide by LNCaP cell homogenates (40 μg) was investigated using 0.2 μCi of [14C]UDPGA in enzymatic assay buffer using 20 μM R1881 or DHT for 16 h of incubation at 37 °C and stopped as described above. The presence of glucuronide was demonstrated on TLC using a migration buffer containing toluene:methanol:acetic acid (7:3:1).

**RNA Isolation, Reverse Transcription (RT), and Real Time PCR**—Total RNA was isolated from LNCaP cells according to the Tri-Reagent acid phenol protocol as specified by the sup-
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### TABLE 1
Conditions for real time PCR

| Genes   | Primers                                    | Final concentration |
|---------|--------------------------------------------|---------------------|
| 28S     | Forward, 5′-AAACTCTGGTGGAGCTCCGT-3′         | 125                 |
|         | Reverse, 5′-CTTACCAGAAATTGGGCCCCACTA-3′    |                     |
|         | Forward, 5′-TTCCGAGAAGCTTCCCTCCCTC-3′      | 125                 |
|         | Reverse, 5′-AACGCGAGTCTGTGTTTTTGC-3′       |                     |
|         | Reverse, 5′-TGAGTCTTCTTCATCCAGTAC-3′       |                     |
|         | Forward, 5′-CCTTCTGGCTTCGCGTCTCC-3′        | 300                 |
|         | Reverse, 5′-ACAGACTGTCGAGCCCCACCC-3′       |                     |
|         | Forward, 5′-TTGGTAAATAGTTCAGCCAGT-3′       |                     |
|         | Forward, 5′-GCATGTCAAGTAATCAGTTG-3′        | 300                 |
|         | Forward, 5′-GCACAAGGTGTTGAAGTCCAAT-3′      |                     |
|         | Forward, 5′-GGCAGGTGCTTGTAGCCTCT-3′        |                     |
|         | Forward, 5′-GGTGAAGTTAACGTTCTGTCC-3′       |                     |
|         | Forward, 5′-CCTTCTGGCTTCGCGTCTCC-3′        |                     |
|         | Reverse, 5′-ACAGACTGTCGAGCCCCACCC-3′       |                     |
|         | Forward, 5′-TTGGTAAATAGTTCAGCCAGT-3′       |                     |
|         | Forward, 5′-GCATGTCAAGTAATCAGTTG-3′        |                     |
|         | Forward, 5′-GCACAAGGTGTTGAAGTCCAAT-3′      |                     |
|         | Forward, 5′-GGCAGGTGCTTGTAGCCTCT-3′        |                     |
|         | Forward, 5′-GGTGAAGTTAACGTTCTGTCC-3′       |                     |
|         | Forward, 5′-CCTTCTGGCTTCGCGTCTCC-3′        |                     |
|         | Reverse, 5′-ACAGACTGTCGAGCCCCACCC-3′       |                     |
|         | Forward, 5′-TTGGTAAATAGTTCAGCCAGT-3′       |                     |
|         | Forward, 5′-GCATGTCAAGTAATCAGTTG-3′        |                     |
|         | Forward, 5′-GCACAAGGTGTTGAAGTCCAAT-3′      |                     |
|         | Forward, 5′-GGCAGGTGCTTGTAGCCTCT-3′        |                     |
|         | Forward, 5′-GGTGAAGTTAACGTTCTGTCC-3′       |                     |
|         | Forward, 5′-CCTTCTGGCTTCGCGTCTCC-3′        |                     |
|         | Reverse, 5′-ACAGACTGTCGAGCCCCACCC-3′       |                     |
|         | Forward, 5′-TTGGTAAATAGTTCAGCCAGT-3′       |                     |
|         | Forward, 5′-GCATGTCAAGTAATCAGTTG-3′        |                     |
|         | Forward, 5′-GCACAAGGTGTTGAAGTCCAAT-3′      |                     |
|         | Forward, 5′-GGCAGGTGCTTGTAGCCTCT-3′        |                     |
|         | Forward, 5′-GGTGAAGTTAACGTTCTGTCC-3′       |                     |
|         | Forward, 5′-CCTTCTGGCTTCGCGTCTCC-3′        |                     |
|         | Reverse, 5′-ACAGACTGTCGAGCCCCACCC-3′       |                     |

### TABLE 2
Sequence of the UGT2B15/B17 siRNA probe and alignment with the sequence of exon 5 from all human UGT2B genes

| UGT2B4   | AAAGTCAGAAGACTTACTCTCTCAAGTCACTGGAAGAGATTA |
| UGT2B7   | AAAGTCAGAAGACTTACTCTCTCAAGTCACTGGAAGAGATTA |
| UGT2B10  | AAAGTCAGAAGACTTACTCTCTCAAGTCACTGGAAGAGATTA |
| UGT2B11  | AAAGTCAGAAGACTTACTCTCTCAAGTCACTGGAAGAGATTA |
| UGT2B15  | CAATGATGCTTCTGAATGATTACCAAGTCACTGGAAGAGATTA |
| UGT2B17  | CAATGATGCTTCTGAATGATTACCAAGTCACTGGAAGAGATTA |
| UGT2B28  | AAAGTCAGAAGACTTACTCTCTCAAGTCACTGGAAGAGATTA |

### RESULTS

**Dose- and Time-dependent Inhibition of UGT2B mRNA Levels Using UGT2B15/B17 siRNA**—The UGT inhibition efficiency was assayed by transfecting LNCaP cells with a non-target (control) or with increasing concentrations of specific UGT2B15/B17 siRNA probe (Table 2) for 72 h. We observed a 70% inhibition of UGT2B15 and -B17 mRNA levels with 25 nM siRNA with no further significant inhibition using higher concentrations (Fig. 1A). Analyses of other UGT2B genes expressed in LNCaP cells reveal that the siRNA probe is specific for UGT2B15 and -B17 mRNA (Fig. 1B). UGT2B7 was also expressed in this cell line, but its levels of expression were almost at the limit of detection (data not shown). Using 25 nM siRNA, we next performed a time-course experiment up to 96 h after transfection. We observed a decrease of 70% in mRNA expression for UGT2B15/B17 at 24 h after transfection with the siRNA, and this inhibition was maintained up to 96 h (Fig. 2A).

**Time-dependent Inhibition of UGT2B15 mRNA and Protein Levels in LNCaP Cells**—To further ascertain the inhibition of UGT2B15/B17 expression, we then quantified the UGT2B15 and -B17 protein levels in transfected cells cultured for 72 and 96 h. As expected, Western blot experiments reveal significant levels of UGT2B15 and -B17 protein in both human liver microsome (positive control) and non-target siRNA-transfected cells. Interestingly, the UGT2B15 protein levels in UGT2B15/B17 siRNA-transfected cells were reduced to an undetectable level after 72 and 96 h, whereas the UGT2B17 protein content was sig-
Inhibition of Androgen-conjugating Activity of UGT2B15/B17 in LNCaP Cells—The consequences of UGT2B15 and -B17 inhibition on the formation of androgen glucuronide were subsequently investigated in glucuronidation assays with siRNA-transfected LNCaP cell homogenates and DHT, ADT, and 3α-DIOL as substrates. The UGT2B17-dependent formation of ADT-3G was 62 and 75%-reduced in UGT2B15/B17-deficient cells cultured for 72 and 96 h, respectively (Fig. 3), which confirms the marked inhibition of UGT2B17 transcript and protein levels. The conjugation of 3α-DIOL and DHT was also decreased by more than 55% (after 72 h) to reach a maximal reduction of almost 95% for 3α-DIOL and 85% for DHT (after 96 h). Because both androgens are substrates of UGT2B15 and UGT2B17, these reductions further confirm that both UGT activities are affected by the UGT2B15/B17 probe (Fig. 3).

These data demonstrate that reduction of UGT2B15 and -B17 expression in LNCaP cells results in a lower androgen-conjugating activity in these cells.

The impact of the loss in UGT2B15/B17 proteins was also assessed in living cells by measuring the concentrations of DHT and DHT-G in media of UGT-deficient and control cells (Fig. 4). In control cells treated with DHT at 1000 and 5000 pM, the amounts of DHT in media after 12 h of incubation were still significant and were dose-dependent as previously demonstrated (30). In the presence of DHT, LNCaP cells also produced DHT-G in a dose-dependent manner. This observation confirms that LNCaP cells in culture are able to glucuronidate physiologically relevant concentrations of DHT. Marked increased amounts of DHT in media were measured in UGT2B15/B17-deficient cells compared with control cells when 1000 and 5000 pm DHT was added for 12 h (Fig. 4A). By contrast, a significant decrease in production of DHT-G was observed (Fig. 4B). Taken together, these observations demonstrated that inhibition of UGT2B15 and -B17 expression significantly reduced but still detectable (Fig. 2B). The equal loading of SDS-PAGE gels was subsequently ensured by Western blotting with the anti-calnexin antibody. These observations confirm that the UGT2B15/B17 siRNA drastically affects the synthesis of the two proteins in LNCaP cells.
reduces the formation of androgen-glucuronide derivatives both in in vitro assay and in cultured cell media and that this inhibition results in higher DHT concentrations in cell media.

**Inhibition of UGT2B15/B17 Enhances the Androgen Signaling Pathway in LNCaP Cells**—To first decipher the consequences of the reduction of DHT glucuronidation on the cellular response to androgens, the mRNA levels of eight androgen-regulated genes were compared in control and UGT-deficient LNCaP cells cultured in the presence or absence of 1 nM concentration of R1881 and DHT for 12 h. As illustrated in Table 3, this set of genes (six up-regulated: PSA, KLK4, NKX3.1, SLC16A6, TMPRSS2, and VEGF; two down-regulated: ABCG1 and RAD23A) was chosen based on previous studies having reported the androgen-dependent regulation of their expression in LNCaP cells (31–43). As expected, the two AR agonists induced PSA, KLK4, NKX3.1, SLC16A6, and TMPRSS2 transcript levels in control cells, whereas VEGF expression was only significantly stimulated by R1881 (Fig. 5, A–F). Interestingly, the DHT-dependent induction of all these genes was significantly higher in cells deficient in UGT2B15/B17 proteins than in control cells. More interestingly, we observed that R1881, but not DHT, significantly decreased ABCG1 and RAD23A expression levels in control cells. In UGT-deficient LNCaP cells, not only the inhibitory effect of R1881 was unaffected, but the DHT treatment reached a significant reduction of ABCG1 and RAD23A mRNA contents (Fig. 5, G and H). These observations indicate that reduction of DHT glucuronidation improves the ability of this androgen to modulate AR target genes expression.

To further address the consequences of reduced androgen glucuronidation on the physiology of LNCaP cells, we then compared the pro-proliferative effects of AR agonists in UGT2B15/B17-deficient and control LNCaP cells. Cells were cultured in the presence of DHT or R1881 for 48 h, and cell proliferation was determined. R1881 treatment provoked 30 and 35% increases in viable cell numbers in control and UGT-deficient LNCaP,
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respectively (Fig. 6A). Interestingly, proliferation in cells treated with 1 nM DHT increased from 10% in control to 35% in UGT2B15/B17 siRNA-transfected cells (Fig. 6A). This result suggests that a reduction of glucuronidation is associated to an increased proliferation of prostate cells in the presence of androgens.

The following experiments were performed to ensure that the results described above reflected a reduction in androgen
glucuronidation and not any other alteration in the androgen signaling pathway. First, we have confirmed that siRNA-mediated inhibition of UGT2B15/B17 does not affect the expression of the androgen receptor (Fig. 7B). Then we also confirmed that LNCaP cells conjugate DHT, but not R1881 (Fig. 7C), which supports that the difference in gene induction and cell proliferation between the two AR activators in UGT-deficient cells reflects the reduced DHT glucuronidation level. Finally, we further confirmed that the increased in PSA gene expression in UGT-deficient cells (Fig. 5) was truly due to an accumulation of the unconjugated androgen. For this purpose cells were treated with DHT, Casodex alone, or both compounds in combination, and PSA mRNA levels were quantified (Fig. 6D). As above, treatment with DHT resulted in a higher PSA induction in UGT2B15/B17-siRNA-transfected cells than in control cells (Fig. 6D). More interestingly, in the presence of Casodex, DHT treatment resulted in an almost complete and identical reduction of the androgen-dependent induction of PSA transcript levels in the two LNCaP cell lines. This last result confirms that the higher effect of DHT observed in UGT2B15/B17-deficient cells reflect its accumulation in these low glucuronidating cells.

DISCUSSION

LNCaP cells contain the necessary machinery to respond to androgens by increasing proliferation and expression of a variety of AR-regulated genes (Fig. 7A) (44, 45). Although a high rate of DHT conjugation is present in LNCaP cells, the DHT response has also been well documented to affect the expression of several genes (17, 26, 46). However, it was intriguing to observe that androgens themselves quickly down-regulate the glucuronidation activity in LNCaP cells as a likely means to avoid inactivation and to facilitate their biological actions (17, 26, 27). Kanaya et al. (47) recently demonstrated that androgen conjugation activity is increased in the LNCaP androgen-independent cell line, and these authors suggested that the alteration of UGT2B15 expression is part of the biological process in the growth of hormone-refractory prostate cancer. Two androgen-conjugating enzymes, UGT2B15 and -B17, are highly expressed in LNCaP cells, and both are responsible for the glucuronidation of DHT and its metabolites 3α-DIOL and ADT (17, 26). In the present study, siRNA against these two enzymes was used to specifically inhibit production of UGT2B15 and -B17 transcripts and, thus, subsequent protein expression and glucuronidation activity. The expression of androgen receptor transcripts was not affected by the siRNA treatment, and the response of several androgen-dependent genes to the agonist R1881 in siRNA-treated cells also ensured that the treatment used to inhibit UGT2B expression did not alter the androgen response machinery. By contrast, when DHT was used as the AR agonist, siRNA treatment resulted in a higher response of AR target genes and increased cell proliferation. Thus, lower androgen-conjugating activity in LNCaP cells resulted in decreased levels of DHT glucuronide formed, leaving more DHT in the media that can then stimulate production of different androgen target genes (Fig. 7B). These results support the concept that glucuronidation in androgen target tissues may influence androgen responses (Fig. 7).

siRNA against UGT2B15 and -B17 enzymes inhibits mRNA levels more than 70% 24 h after transfection, and this inhibitory
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FIGURE 6. Loss of UGT2B15/B17 in LNCaP cells enhances cell proliferation (A) without affecting the expression of the androgen receptor (B), and whereas LNCaP cells fail to glucuronidate R1881 (C), the AR antagonist Casodex blocks the DHT-dependent induction of PSA in both control and UGT-deficient cells (D). A, 72 h after transfection with control or the UGT2B15/B17 siRNA probe, media containing 0.2% fetal bovine serum with or without 1 nM DHT were added to cells for an additional 48 h. Media were supplemented with 20 μl of CellTiter 96 Aqueous One Solution Cell Proliferation Assay for 2 h, and optical density at 490 nm was read. B, 96 h after transfection with siRNA probes, mRNA levels of the androgen receptor were analyzed by real-time PCR. Data are the mean of two experiments in triplicate. Statistical differences between two groups: *, p ≤ 0.05. NS, not significant.

FIGURE 7. Representation of the cellular impact of UGT2B15/B17 siRNA in androgen-dependent LNCaP cells. LNCaP cells transfected with a Non-target siRNA express high levels of androgen-conjugating UGT2B enzymes which inactivate a large amount of DHT (A). By contrast, siRNA-mediated repression of UGT2B15 and -B17 protein expression results in increased intracellular concentrations of DHT. Thus, the active androgen remains available to stimulate the AR-mediated gene regulation, as observed in Fig. 5 with AR target genes (B).

effect was still observed 96 h after transfection. Analyses of the other UGT2B genes in LNCaP cells revealed that the siRNA is highly specific for UGT2B15 and -B17 with an inhibition less than 20% after 96 h for the UGT2B10 and B11 mRNA levels. These two transcripts have ~70–75% nucleotide homology with UGT2B15/B17, and the corresponding proteins do not conjugate androgens (25, 48). The difference in two nucleotides of the 16 used may explain the small inhibition effect observed (Table 2). We also observe an important reduction of UGT2B15 protein levels but a less significant inhibition in B17 protein 96 h after siRNA transfection, suggesting that UGT2B17 is more stable than UGT2B15 in LNCaP cells. Surprisingly, previous reports obtained in stably transfected UGT2B15 and -B17 in HEK293 cells indicate that UGT2B17 protein had a half-life of 3 h compared with 24 h for UGT2B15 protein (12). It is possible that the difference between the two cell lines, HK293 and LNCaP cells, may be responsible for these changes in protein stability, or over-expression of UGT2B proteins in HK293 could modify the stability of these proteins. Our data indicate that this slight difference in protein levels between UGT2B15 and -B17 has, however, no major impact on glucuronidation since for all androgen substrates tested a marked decrease is observed. Indeed, the levels of ADT-glucuronide formed, a specific substrate of UGT2B17, are quite comparable with that obtained with DHT-G and 3α-Diol-17G, two products produced by both enzymes. When only UGT2B15 was inhibited using specific siRNA (data not shown), we detected only a small but significant inhibition in glucuronidation of androgens, suggesting that both UGT2B15 and -B17 need to be targeted to obtain maximal inhibitory effects.

To assess the impact of UGT2B15 and -B17 in the regulation of intracellular concentration of androgens, we analyzed the expression of several androgen-target genes that were shown to be up- or down-regulated using microarray in LNCaP cells (Table 3). Two of these genes, PSA and NKX3.1, are well established genes to study the activity of AR in LNCaP cells (36, 44–46, 49, 50). Recently, Cheng et al. (51) reported that silencing AR expression in LNCaP cells decreased the activity of a reporter luciferase construct of the human PSA gene. This suggested that a lower amount of AR reduced the activation of androgen-target gene PSA. The present data obtained with LNCaP cells deficient in UGT2B15 and -B17 proteins show that lower androgen-conjugating activity induces an increase in media and in intracellular concentra-
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In conclusion, we produced LNCaP cells deficient in UGT2B15 and -B17, and 3α-DIOL-3-glucuronide, which is a specific product of B7. Overall, these observations suggest that UGT2B15 may be the major UGT2B responsible for the production and secretion of 3α-DIOL-17G in vivo.

In further support to the importance of UGT2B15 in the prostate is a recent series of genotyping studies which reports that individuals with the low androgen-glucuronidating UGT2B15D85 allele, in comparison to that of the UGT2B15Y85 allele, have statistically significant higher risks of having prostate cancer (21–24). A previous study has shown that the conjugating activity of UGT2B15Y85 toward DHT and 3α-DIOL is significantly higher than that for UGT2B15D85, and it was suggested that such a difference may affect the prostate concentrations of bioactive DHT (11). The UGT2B15 transcript and protein have been detected in the luminal epithelial cells of the prostate where AR is also predominantly localized (12, 20, 56). Those luminal cells are also implicated in prostate cancer. Based on the hypothesis that higher concentrations of intraprostatic DHT for a long period of time may influence androgen-dependent genes and cell proliferation, it was suggested that the presence of UGT2B15D85 may increase the risk of prostate cancer. The present in vitro data using LNCaP cells strongly support this hypothesis.

In conclusion, we produced LNCaP cells deficient in UGT2B15 and -B17 proteins, and we demonstrated the impact of this loss on androgen glucuronidation. The increase in bioactive DHT concentrations influences androgen-dependent gene expression and cell proliferation. These data support the concept that inactivation of androgen by UGT2B enzymes in tissues is an important means to regulate the action of androgen.

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