Distinct Patterns of Dysregulated Expression of Enzymes Involved in Androgen Synthesis and Metabolism in Metastatic Prostate Cancer Tumors

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Abstract
Androgen receptor (AR) signaling persists in castration-resistant prostate carcinomas (CRPC), because of several mechanisms that include increased AR expression and intratumoral androgen metabolism. We investigated the mechanisms underlying aberrant expression of transcripts involved in androgen metabolism in CRPC. We compared gene expression profiles and DNA copy number alteration (CNA) data from 29 normal prostate tissue samples, 127 primary prostate carcinomas (PCa), and 19 metastatic PCas. Steroidogenic enzyme transcripts were evaluated by quantitative reverse transcriptase PCR in PCa cell lines and circulating tumor cells (CTC) from CRPC patients. Metastatic PCas expressed higher transcript levels for AR and several steroidogenic enzymes, including SRD5A1, SRD5A3, and AKR1C3, whereas expression of SRD5A2, CYP3A4, CYP3A5, and CYP3A7 was decreased. This aberrant expression was rarely associated with CNAs. Instead, our data suggest distinct patterns of coordinated aberrant enzyme expression. Inhibition of AR activity by itself stimulated AKR1C3 expression. The aberrant expression of the steroidogenic enzyme transcripts was detected in CTCs from CRPC patients. In conclusion, our findings identify substantial interpatient heterogeneity and distinct patterns of dysregulated expression of enzymes involved in intratumoral androgen metabolism in PCa. These steroidogenic enzymes represent targets for complete suppression of systemic and intratumoral androgen levels, an objective that is supported by the clinical efficacy of the CYP17 inhibitor abiraterone. A comprehensive AR axis–targeting approach via simultaneous, frontline enzymatic blockade, and/or transcriptional repression of several steroidogenic enzymes, in combination with GnRH analogs and potent antiandrogens, would represent a powerful future strategy for PCa management. Cancer Res; 72(23); 6142–52. ©2012 AACR.

Introduction
Gonadal androgen depletion and/or blockade have been the standard first-line systemic treatment for advanced prostate cancer (PCa) for the past 7 decades, producing declines in prostate-specific antigen (PSA) and tumor regression. Despite peripheral androgen levels in the castrate range, eventual regrowth occurs as a castration-resistant PCa (CRPC) and is invariably lethal (1). The androgen receptor (AR) signaling axis remains active in most CRPCs, as evidenced by the frequent reexpression of AR-target genes such as PSA and TMPRSS2. The AR axis thus represents an important therapeutic target, a concept that has been validated in recent clinical trials of second-line hormonal manipulations with abiraterone acetate, a CYP17 inhibitor that blocks steroid biosynthesis (2–7), and MDV3100 (enzalutamide), a new antiandrogen (5, 8–11). Several mechanisms that allow AR activation despite castrate levels of peripheral testosterone have been reported in CRPC, including the persistence of residual intratumoral androgens at concentrations sufficient to activate AR (12–19). Compared with primary prostate tumors or normal prostate tissue, CRPC displays upregulated expression of several transcripts encoding for enzymes involved in androgen metabolism (18, 20–23).

The clinical relevance of the above findings is validated by the activity of the CYP17 inhibitor abiraterone (2–7), recently shown to prolong overall survival in chemotherapy-treated CRPC patients, and now U.S. Food and Drug Administration-approved for this indication. The responses, however, are incomplete and all tumors eventually progress with resumed PSA expression, an indication of reactivation of AR signaling.
Preliminary evidence suggests that abiraterone-resistant PCAs overexpress CYP17A1 and other steroidogenic transcripts (including STAR, CYP11A1, HSD3B1, and AKR1C3; ref. 24), suggesting maintenance of capacity for in situ steroidogenesis as a potential mechanism of treatment failure. Additional data suggest that intratumor CYP11-dependent pregnenolone/progesterone synthesis can contribute to resistance to abiraterone (25) and strengthen the notion that CRPCs resistant to CYP17 inhibition may remain ligand dependent and AR dependent, and, therefore, responsive to therapies that can further suppress de novo intratumoral steroid synthesis (25). We hypothesized that the delineation of the mechanisms leading to dysregulated expression of androgen metabolism enzymes would provide important insight into possible mechanisms of resistance to abiraterone, and would help identify additional targets in this pathway and facilitate rational design of future drug combinations for clinical trials in CRPC as candidate components of a comprehensive AR axis–targeting approach.

Toward that aim, we mined datasets from a recently reported comprehensive-integrated oncogenomic analysis of banked tissue samples from primary and metastatic prostate PCAs and normal prostate controls (26) to define the frequency of alterations in androgen metabolism pathways. We found aberrant expression for several of these steroidogenic enzymes and investigated mechanisms accounting for this phenomenon.

Materials and Methods

PCa tissue specimens and oncogenomic profiling

The methodology for our integrated analysis of transcriptomes and CNAs in PCa has been reported previously (26). Briefly, gene expression profiles of 29 normal prostate tissue samples, 131 primary PCAs, and 19 metastatic (8 noncastrate, 11 castrate) PCAs were generated using Affymetrix Human Exon 1.0 ST arrays. Data from 4 primary tumor samples were excluded from analysis because of prior neoadjuvant hormonal or chemotherapy treatment. Expression outliers, defined as transcripts with significant up- or downregulation in that particular specimen compared with the distribution of expression for that transcript in normal prostate samples, were determined as previously (26, 27). In this nonparametric approach, an empirical distribution function generated from transcript expression in the 29 normal prostate tissues was used to transform expression in the tumor samples, from which outliers were determined with the criteria described in the Benjamini and Hochberg algorithm (28) at an error rate \( a = 0.01 \) (26).

Copy-number alterations (CNA) were assessed with Agilent 24K array comparative genomic hybridization (aCGH) microarrays (described in detail in ref. 26). All patients provided informed consent. Samples were procured and the study was conducted under Memorial Sloan-Kettering Cancer Center Institutional Review Board approval. Clinical and pathologic data were entered and maintained in a prospective PCa database.

The complete data are freely available through a web-based portal (29). The full raw data are available via GEO (accession no. GSE21032).

List of studied transcripts

We studied transcripts for enzymes participating in androgen synthesis and metabolism (Fig. 1A and Supplementary Table S1). We also used a previously published AR-dependent transcript signature (30) and applied it to our gene expression data to quantify AR axis signaling output.

In vitro treatment of PCa cells

PCa LNCaP cells (purchased from American Type Culture Collection and passaged for fewer than 6 months) were grown in RPMI-1640 medium supplemented with 10% FBS (Omega Scientific). For androgen deprivation, the cells were incubated in RPMI-1640 medium supplemented with 10% charcoal-stripped FBS (CSS, Omega Scientific) for 48 hours. R1881 (NEN Life Science Products) was used at 1 nmol/L. The novel antiandrogen MDV3100 (enzalutamide; Medivation; refs 8, 9) was used at 10 μmol/L. Quantitative reverse transcriptase PCR (RT-PCR) analysis for steroidogenic enzyme expression was conducted using a StepOne Plus instrument and Taqman probes (both from Applied Biosystems).

Quantitative RT-PCR analysis of CTCs from CRPC patients for expression of AR, KLK3 (PSA), and steroidogenic enzymes

Circulating tumor cells (CTC), defined as EpCaM(+), CD45(−) events, were collected by fluorescence-activated cell sorting (FACS, MoFlo; Beckman Coulter), using empirically defined gates based on healthy volunteer samples spiked with (positive control) or without (negative control) PCa cells (LNCaP cells). Ten milliliters of blood from patients with CRPC, obtained with the patients’ informed consent under a Memorial Sloan-Kettering Cancer Center Institutional Review Board-approved protocol were collected into an EDTA tube (BD Biosciences) and processed within 5 hours of blood draw. Mononuclear cells were isolated via density gradient centrifugation (Ficoll-Paque PLUS; GE Healthcare Biosciences) and labeled with conjugated antibodies EpCaM-PE and CD45-APC (Miltenyi Biotec Inc.). EpCaM(+) /CD45(−) /DAPI(+) events were sorted into 1-Step RT-PCR mix (Invitrogen) for primer-specific multiplex reverse transcription (using Universal PCR Master Mix, Applied Biosystems) and 14 cycle “preamplification” PCR using Taqman probes (Applied Biosystems). Standard 40-cycle quantitative PCR was then conducted on the 96 × 96 BioMark chip (Fluidigm).

Statistical analysis

The average expression of each transcript of interest was compared between normal prostate tissue, primary PrCa, and metastatic PrCa using 1-way ANOVA and the LSD, Bonferroni, and Dunnett C post hoc tests. Two-sided \( t \) tests were used to compare the number of “proandrogenic” or “antiandrogenic” transcripts that are differentially expressed in metastatic versus primary carcinomas. \( x^2 \) tests were used to compare the number of cases of metastatic versus primary carcinomas that had differential expression of at least 1 proandrogenic or antiandrogenic transcript. The linear correlation between various individual (or groups of) mRNAs was evaluated by calculation of the Pearson correlation coefficient.
Figure 1. Pathways of testosterone/DHT biosynthesis and metabolism, associated enzymes and their expression in metastatic PCa specimens. A, cholesterol, the precursor of all steroidogenesis, is converted to DHT via several enzymatic steps: in the Δ5 pathway (named after the presence of a double carbon bond in the C5 position of the A steroid ring; steroids highlighted in green) and the Δ4 pathway (steroids highlighted in light red), testosterone is synthesized and then reduced by 5α-reductases to DHT, which has a approximately 5- to 10-fold higher affinity for AR. Androgen precursors can also be reduced before testosterone synthesis, generating an alternate pathway (backdoor pathway, steroids highlighted in light blue) that bypasses testosterone and leads to DHT. This pathway has been proposed to be active in prostate tissue, in particular PCa (17). Recently, it was shown that the dominant route of DHT synthesis in CRPC bypasses testosterone (23), and instead requires 5α-reduction of androstenedione by SRD5A1 to 5α-androstanedione (highlighted in yellow), which is then converted to DHT. Testosterone and DHT are oxidized (via cytochrome P450 3A oxidases) followed by conjugation to glucuronides (via uridine diphospho-glucuronosyl transferases UGT2B7, UGT2B15, and UGT2B17) that are then excreted. Enzymes involved in promoting testosterone/DHT synthesis are highlighted in green, whereas enzymes promoting their metabolism/inactivation are highlighted in dark blue. The target sites of clinically relevant inhibitors are also shown (figure modified from ref. 19). B, heatmap of outliers (red: overexpressed transcript, blue: underexpressed transcript) for AR and transcripts involved in androgen metabolism in the metastatic PCa specimens (outlier expression compared with the distribution of expression in normal prostate samples; see Materials and Methods and ref. 26).
Results

High interpatient variability of dysregulated expression of individual transcripts involved in androgen metabolism in PCa

Expression of our panel of transcripts encoding for enzymes involved in androgen synthesis and metabolism (Fig. 1A and Supplementary Table S1) was analyzed for outliers (overexpressors or underexpressors) and revealed high interpatient variability, with several specific transcripts highly over- or underexpressed in nearly all samples and others altered only in a minority of tumors (results are presented in Figs 1B and 2, for metastatic and primary tumors, respectively). Average mRNA levels for each transcript in each group are presented in Supplementary Table S2. Results from 1-way ANOVA comparing average expression of each transcript between groups (normal, primary PrCa, and metastatic PrCa), as well as the respective P values, are presented in Supplementary Table S3.

We found increased average expression of AR, AKR1C3, SRD5A1, and SRD5A3, and decreased average expression of SRD5A2, CYP3A4, CYP3A5, and CYP3A7 in metastatic PrCa (boxplots for log2-based mRNA expression are shown in Fig. 3). Importantly, several other transcripts were dysregulated in smaller subsets of tumors (suggesting potential contribution to activation of the androgen AR axis in those particular tumors), whereas not reaching statistical significance on average among all tumors in our panel. This key finding raises the hypothesis that increased intratumoral androgens may be caused by dysregulation of different enzymes in different tumors.

For the transcripts that are expected to have a proandrogenic effect (i.e., increase ligand availability and, thus, AR activity; Group A in Supplementary Table S1), we found that metastatic carcinomas overexpressed, on average, 4.7 transcripts (range 1–13, SD 2.7), compared with 1.7 transcripts in the primary carcinomas (range 0–11, SD 2.0, 2-sided t test, P = 0.00024). All (19/19) metastatic carcinomas overexpressed at least one such transcript, compared with 92/127 for primary carcinomas (χ² P < 0.01). For the antiandrogenic transcripts (i.e., enzymes overall associated with androgen degradation/inactivation and, thus, expected to decrease AR activity; Group B in Supplementary Table S1), the metastatic carcinomas underexpressed, on average, 3.5 transcripts (range 0–9, SD 2.2), compared with 2.6 transcripts for the primary carcinomas (range 0–9, SD 2.0, 2-sided t test, P = 0.0128). Moreover, 18/19 metastatic carcinomas underexpressed at least one such transcript, compared with 102/127 for primary carcinomas (χ² P = 0.125). Thus, consistent with the data in Fig. 1B, metastatic tissues exhibited significantly more variable expression patterns than primary carcinoma or normal prostate tissue.

Association of expression of transcripts involved in androgen metabolism with AR transcriptional output

We investigated whether the variability in expression of these steroid-related transcripts leads to enhanced AR signaling output. We calculated a composite steroid enzyme expression as the sum of the proandrogenic transcripts (that overall are associated with steroid synthesis and increased AR activity) minus the sum of the antiandrogenic transcripts (that overall are involved in androgen degradation/inactivation and, thus, expected to decrease AR activity), and found that there was a positive correlation with the AR transcriptional output signature in our metastatic specimen panel (Pearson correlation coefficient R² = 0.43, P = 0.0022, Supplementary Fig. S1A). The statistical significance persisted even when the AR transcript itself was removed from the analysis (R² = 0.36, P = 0.0061, Supplementary Fig. S1B).

Gene CNAs appear not to be the cause of dysregulated expression of transcripts involved in androgen metabolism in PCa

We integrated the CNAs identified in ref. (26) with our transcriptome data, to assess the role of genomic alterations on the steroid metabolism axis in our PCa specimens. The histograms in Figs 4 (metastatic carcinomas) and 5 (primary carcinomas) show the fraction of outliers for each transcript superimposed with the fraction of samples exhibiting CNA. With the exception of very few transcripts (e.g., CYP11B1 in Fig. 4), only a minority of the specimens with altered mRNA expression (over- or underexpressor outliers) had corresponding gene copy gains or losses that could account for the dysregulated mRNA levels. Thus, for most of these genes, transcriptional regulation, rather than altered gene copy number, is the likely cause of dysregulated expression.

Patterns of coordinated expression of transcripts involved in androgen metabolism in PCas suggest distinct regulatory mechanisms

As our results suggested that the dysregulation of androgen-related transcripts in PCas occurs at the transcriptional level, we assessed for similarities in the pattern of their expression that might indicate the existence of common regulatory mechanisms. Using linear correlation analysis of the log2-based mRNA levels in primary and metastatic carcinomas, we identified 4 distinct groups of transcripts with highly coregulated patterns of expression (Supplementary Table S4): Group 1: CYP11A1, CYP11B1, CYP11B2, CYP17A1, CYP19A1, CYP21A2, HSD3B1, HSD3B2, HSD3B7, STAR, and UGT2B15; Group 2: AKR1C1, AKR1C2, AKR1C3, and AKR1C4; Group 3: CYP3A4, CYP3A5, and CYP3A7; and Group 4: UGT2B15 and UGT2B17. These 4 groups suggest respective distinct patterns of (dys)regulation of expression of enzymes involved in androgen metabolism in PCas. Group 1 includes most enzymes expressed in the adrenals and necessary for conversion of cholesterol to adrenal androgen precursors (DHEA and androstenedione). Group 2 is the AKR1C family of enzymes, which, among other functions, can convert adrenal androgens to testosterone. The Group 3 enzymes are involved in phase I of DHT inactivation (oxidation), whereas Group 4 enzymes catalyze phase II of DHT inactivation (glucuronidation). For more details on the role of these enzymes in androgen metabolism, please see Fig. 1A.

The enzymes of the AKR1C family are negatively regulated by androgen

We next investigated the regulation of the Group 2 transcripts, that is, the AKR1C family enzyme transcripts, because
Figure 2. Heatmap of outliers (red, overexpressed transcript; blue, underexpressed transcript) for AR and transcripts involved in androgen metabolism in the primary PCa specimens (outlier expression compared with the distribution of expression in normal prostate samples; see Materials and Methods and ref. 26).
AKR1C3 plays a crucial role in conversion of DHEA and androstenedione to testosterone. We mined our transcriptome data (from primary and metastatic tumors) for transcripts highly coregulated with AKR1C3. Not surprisingly, we found the other family members, AKR1C1, AKR1C2, and AKR1C4, to be coregulated (Supplementary Table S5). Transcripts highly negatively associated with AKR1C3 were KLK3, ACPP, ABCC4, KLK2, and other AR-driven transcripts (Supplementary Table S5). These findings suggested that high AKRIC family enzyme expression is inversely associated with AR activity. This was confirmed in our transcriptome data from metastatic specimens, where the AR transcriptional output (quantified using an AR-dependent gene signature previously derived by treating the LNCaP PCa cell line with androgen for 24 hours; ref. 30) was inversely associated with expression of each individual AKRIC family enzyme (Fig. 6A).

Figure 3. Boxplots of average mRNA expression (log2-based) for AR, AKR1C3, SRD5A1, SRD5A3, SRD5A2, CYP3A4, CYP3A5, and CYP3A7 in normal prostate tissue, primary PCas, and metastatic PCas. We found increased expression of AR, AKR1C3, SRD5A1, and SRD5A3, and decreased expression of SRD5A2, CYP3A4, CYP3A5, and CYP3A7 in metastatic PrCa. **, P < 0.01 versus both normal tissue and primary carcinomas; *, P < 0.01 versus normal tissue. Complete results are presented in Supplementary Table S3.
Suppression of gonadal androgen synthesis does not achieve complete ablation of androgen signaling in the prostate microenvironment. Even when circulating testosterone is confirmed to be at castrate levels, intratumoral androgens persist at levels sufficient to activate AR (12–17). CRPC can locally convert adrenal precursors to more active androgens (testosterone and DHT; refs 31, 32). Moreover, de novo steroidogenesis in CRPC, using cholesterol as a precursor, has been supported by some (18, 22, 33), but not all studies (21). In the present study, we investigated the mechanism(s) leading to aberrant expression of enzymes involved in steroid metabolism in CRPC. Using data from an integrated oncogenomic analysis of primary and metastatic specimens (26), we documented that metastatic PCAs express higher average transcript levels for AR and several steroidogenic enzymes, including SRD5A1, SRD5A3, and AKR1C3, whereas expression of SRD5A2, CYP1A4, CYP1A5, and CYP1A7 is decreased, compared with normal prostate tissue or primary prostate carcinoma. Collectively, these data show that...
CRPC cells have increased expression of AR and steroidogenic enzymes, and decreased expression of enzymes that can inactivate DHT (CYP3A4, CYP3A5, or CYP3A7), a state that is predicted to increase in situ androgen levels and enhance AR activation. This was supported by the finding of positive correlation between the composite enzyme expression and the AR transcriptional signaling output signature (a measure of AR activation) in our samples.

Moreover, we found high interpatient variability of expression of individual transcripts in primary and metastatic PCas, suggesting that, within individual tumors, activation of the androgen synthesis axis may occur at various levels and by various routes, but with a predicted common end result, that is, increased tissue androgen levels and stimulation of AR. Such a result, which validates the androgen synthesis pathway en bloc as a mechanism of CRPC cell survival and resistance to androgen deprivation, should not be surprising, considering the vast heterogeneity observed in other oncogenic signaling pathways even within the same tumor (34), but may complicate targeting at the individual patient level. For example, while the predominant form of 5α-reductase in normal prostate is the type-2 (SRD5A2), in most PCas the relative expression pattern of the 2 enzymes is inverted, with increased expression of the type 1 (SRD5A1) and decreased expression of the type 2 enzyme. In clinical practice, this suggests that dutasteride, a dual 5α-reductase inhibitor (35), should be the preferred agent to target this enzymatic step in CRPC, rather than finasteride, which is relatively selective for the type 2 enzyme.

Figure 6. Expression of the AKR1C family members is inversely related to androgen signaling. A, inverse correlation between expression levels of AKR1C1, AKR1C2, AKR1C3, and AKR1C4 versus an AR-regulated gene signature (indicative of AR signaling output) in metastatic PCas. All P values less than 0.001. B, exposure of LNCaP cells to androgen deprivation (medium supplemented with 10% charcoal-stripped serum, CSS) for 48 hours potently upregulates expression of AKR1C3. This upregulation is suppressed by addition of the synthetic androgen R1881 (1 nmol/L). Moreover, treatment of LNCaP cells (growing in medium supplemented with 10% regular FBS) with the antiandrogen enzalutamide (MDV3100) upregulates AKR1C3 expression. AKR1C3 mRNA levels were quantified by qRT-PCR, normalized to actin mRNA levels, and expressed as a percentage over values of control wells (grown in medium supplemented with 10% regular FBS) ± SD (*, P < 0.05; **, P < 0.005).
Furthermore, in the era of personalized medicine, this interpatient heterogeneity in intracrine metabolic pathways raises the question whether real-time profiling of a patient’s tumor cells may provide predictive biomarkers of sensitivity to androgen synthesis inhibitors and even guide a more focused treatment approach by targeting the specific overexpressed enzyme. Although we have not conducted a conclusive study, our preliminary data show that expression of these steroidogenic enzymes is detectable in CTCs in these tumors and providing a noninvasive method for monitoring of their expression. Results are presented as cycle threshold (Ct) values (i.e., the number of cycles required for the fluorescent signal to cross a previously defined threshold) in a heatmap. Ct values are inversely proportional to the amount of target nucleic acid in the sample. Therefore, low Ct values (orange or even yellow color) indicate strong expression of the target mRNA, whereas high Ct values (e.g., dark blue color) indicate weak expression. Each sample was run in duplicate. VCaP cells served as a positive control.

![Figure 7](image.png)

Figure 7. Multiplex qRT-PCR analysis of CTCs from CRPC patients for AKR1C3, SRD5A1, CYP17A1, AR, and KLK3 (PSA) transcripts reveals positivity in several CTC samples, confirming that these transcripts are expressed in the cancer cells in these tumors and providing a noninvasive method for monitoring of their expression. Results are presented as cycle threshold (Ct) values (i.e., the number of cycles required for the fluorescent signal to cross a previously defined threshold) in a heatmap. Ct values are inversely proportional to the amount of target nucleic acid in the sample. Therefore, low Ct values (orange or even yellow color) indicate strong expression of the target mRNA, whereas high Ct values (e.g., dark blue color) indicate weak expression. Each sample was run in duplicate. VCaP cells served as a positive control.

platform for identification and exploration of biomarkers that may guide patient eligibility for clinical trial enrollment and may serve as a potential basis for individualized therapy, possibly predicting drug efficacy and evaluating mechanism(s) of resistance.

The aberrant expression patterns of androgen axis transcripts were only rarely associated with respective CNAs in our cohort, suggesting that this dysregulation occurs mainly at the mRNA level. Analysis of these expression patterns identified distinct groups with highly coregulated expression. One group of transcripts, comprising aldol-keto reductase family 1 C (AKR1C1 through 4), was found to be inversely correlated to AR transcriptional activity, as reflected by an AR-dependent gene signature (30). This suggested that the expression of the AKR1Cs is suppressed by androgen. We confirmed that both androgen deprivation and an AR antagonist induce AKR1C3 expression. The AKR1C1–4 genes are located on chromosome 10p15 in tandem, sharing more than 86% amino acid sequence identity (37). Our findings suggest that androgen deprivation triggers a feedback loop that enhances the ability of PCa cells to metabolize adrenal precursors into testosterone and DHT, thus sustaining tissue androgen levels. Evidence for such a feedback loop was recently reported in patients with CRPC, where treatment with the AR antagonist enzalutamide resulted in increased bone marrow testosterone levels (41). Moreover, abiraterone-resistant PCa xenografts overexpress several steroidogenic enzymes, including AKR1C3 (24). This proposed adaptation/survival mechanism is also supported by the finding that, after gonadal androgen suppressive therapy, intraprostatic androgen levels persist at approximately 25% of baseline (whereas serum androgen levels decrease to ~7.5% of baseline) and are no longer correlated with the serum level of testosterone, but with serum levels of the adrenal precursors DHEA and DHEA-S (12, 13, 42). This suboptimal suppression of intratumoral androgens may allow for the survival of cancer cells that will eventually lead to CRPC. Indeed, the rate of pathologic complete response in prostatectomy specimens removed after 3 to 8 months of neoadjuvant androgen deprivation therapy is less than 3% (43). Collectively, these findings support our hypothesis that the almost universal persistence of PCa cells after gonadal androgen suppression, and the eventual emergence of CRPC, is facilitated by adaptive cellular changes that occur very early after initiation of gonadal suppression and allow PCa cells to maintain adequate intratumoral androgen levels and survive despite peripheral castrate androgen levels. A more comprehensive AR axis targeting at multiple levels (androgen synthesis, metabolism, and action) and at all relevant sites (gonadal, adrenal, intratumoral) simultaneously at the time of initiation of endocrine therapy, aiming at maximal frontline inhibition of the AR axis, is warranted, instead of the current treatment paradigm of sequentially adding agents at the time of disease progression (44). Clinically, our hypothesis can be tested in trials incorporating abiraterone and/or enzalutamide at the time of initiation of GnRH analog therapy in the neoadjuvant or metastatic setting. Preliminary observations support the promise of this approach (45). Furthermore, AKR1C3 inhibitors (37–40) would also be interesting choices to be tested concurrently with GnRH analogs. A recently
reported bifunctional inhibitor of both AKR1C3 and AR represents an intriguing paradigm (46).

An obvious limitation of our study is that, because of the retrospective nature of the analysis, direct measurement of androgen levels in these tissues could not be conducted. Consequently, the correlation between mRNA levels and enzymatic activity cannot be confirmed in this study.

In summary, our comprehensive integrated oncogenic approach identified aberrant expression of enzymes involved in androgen synthesis and metabolism that may lead to increased transcriptional output of the AR axis in CRPC. It is likely that the interpatient variations in these intracrine pathways of steroid metabolism can be evaluated by noninvasive, real-time monitoring of expression in CTCs and could serve as potential basis for individualized therapy. Collectively, these findings further support the notion that the AR axis is still a very important target in CRPC, and that, despite gonadal suppression, prostate tumors may not encounter (yet) a completely androgen-free microenvironment (47). The clinical activity of the CYP17 inhibitor abiraterone (2, 6, 7) validates the importance of this pathway in CRPC. As inhibitors of AR (enzalutamide, ARN509; refs 8, 9, 11, 48), CYP17 (abiraterone; ref. 4), AKR1C3 (37–40, 46), and SRD5A1 (dutasteride; ref. 35) are already available or in clinical development, we propose that frontline maximal suppression of the AR axis with combination therapy targeting simultaneously multiple components of this axis may enhance antitumor activity (44).

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

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