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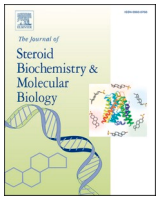

Prostate cancer androgen biosynthesis relies solely on CYP17A1 downstream metabolites

Gido Snaterse [a,1], Angela E. Taylor [b,1], J. Matthijs Moll [c,1], Donna M. O'Neil [b], Wilma J. Teubel [c], Wytske M. van Weerden [c], Wiebke Arlt [b,d,e,2], Johannes Hofland [a,b,*,2]

[a] Section of Endocrinology, Department of Internal Medicine, Erasmus MC, Rotterdam, the Netherlands
[b] Institute of Metabolism and Systems Research, University of Birmingham, Birmingham, United Kingdom
[c] Department of Urology, Erasmus MC, Rotterdam, the Netherlands
[d] Institute of Clinical Sciences, Imperial College London, London, United Kingdom
[e] MRC Laboratory of Medical Sciences, London, United Kingdom

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ABSTRACT

Prostate cancer (PC) is dependent on androgen receptor (AR) activation by testosterone and 5α-dihydrotestosterone (DHT). Intratumoral androgen accumulation and activation despite systemic androgen deprivation therapy underlies the development of castration-resistant PC (CRPC), but the precise pathways involved remain controversial. Here we investigated the differential contributions of de novo androgen biosynthesis and androgen precursor conversion to androgen accumulation. Steroid flux analysis by liquid chromatography-tandem mass spectrometry (LC-MS/MS) was performed on (CR)PC cell lines and fresh patient PC tissue slices after incubation with classic and alternative biosynthesis intermediates, alongside quantitative PCR analysis for steroidogenic enzyme expression. Activity of CYP17A1 was undetectable in all PC cell lines and patient PC tissue slices. Instead, steroid flux analysis confirmed the generation of testosterone and DHT from adrenal precursors and reactivation of androgen metabolites. Precursor steroids upstream of DHEA were converted down the first steps of the alternative DHT biosynthesis pathway, but did not proceed through to active androgen generation. Comprehensive steroid flux analysis of (CR)PC cells provides strong evidence against intratumoral de novo androgen biosynthesis and demonstrates that androgen precursor steroids downstream of CYP17A1 activities constitute the major source of intracrine androgen generation.

1. Background

Prostate cancer (PC) growth is primarily driven by activation of the androgen receptor (AR) through its canonical ligands testosterone and 5α-dihydrotestosterone (DHT). Consequently, metastatic PC is treated with androgen deprivation therapy (ADT) with LHRH a(nta)gonists or orchidectomy [1]. Continued AR activation despite castrate levels of androgens significantly contributes to disease progression after ADT [2, 3]. Inhibition of this pathway underpins the success of anti-hormonal therapy with steroidogenic enzyme inhibitors and next-generation anti-androgens. Abiraterone acetate, which inhibits the key androgen biosynthesis enzyme cytochrome P450 17α-hydroxylase/17,20-lyase (CYP17A1), and potent anti-androgen, such as enzalutamide, prolong progression-free survival in castration-resistant prostate cancer (CRPC), both before and after docetaxel chemotherapy [4–7]. Novel anti-androgens have also shown superior efficacy as add-on first-line

Abbreviations: 3α-diol, 3α-androstanediol; 5α17HP, 5α-pregnan-17-ol-3,20-dione; 5αP, 5α-pregnan-3,20-dione; 5-diol, androstenediol; 17alloP, 17α-hydroxyallopregnanolone; 17OHPreg, 17α-hydroxypregnenolone; 17OHProg, 17α-hydroxyprogesterone; Adione, androstenedione; ADT, androgen deprivation therapy; alloP, allopregnanolone; AR, androgen receptor; AST, androsterone; CRPC, castration-resistant PC; CT, Threshold cycles; CYP17A1, cytochrome P450 17α-hydroxylase/17,20-lyase; DCC, dextran-coated charcoal-stripped; DHEA, dehydroepiandrosterone; DHT, 5α-dihydrotestosterone; HSD, hydroxysteroid dehydrogenase; LC-MS/MS, liquid chromatography-tandem mass spectrometry; NAP, normal adjacent prostate; PC, prostate cancer; PGM, prostate growth medium; Preg, pregnenolone; Prog, progesterone; T, testosterone; TURP, transurethral resection of the prostate.

* Correspondence to: Section of Endocrinology, Department of Internal Medicine, Erasmus MC, Doctor Molewaterplein 40, Rotterdam 3015 GD, the Netherlands.
E-mail address: j.hofland@erasmusmc.nl (J. Hofland).

[1] Shared first authors
[2] Shared senior authors

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therapy to ADT in PC patients [8,9]. Their ability to prolong patient survival confirms the relevance of intratumoral androgens of non-testicular origin that activate the AR in PC cells.

The residual presence of intratumoral androgens following ADT is hypothesized to come from two sources, namely intracrine conversion of circulating androgen precursors or de novo steroidogenesis within PC cells. The crucial androgen precursor dehydroepiandrosterone (DHEA) is generated from 17α-hydroxypregnenolone via CYP17A1 17,20 lyase activity, mainly in the adrenal gland. DHEA and its downstream metabolite androstenedione can be utilized for intra-tumoral conversion into testosterone and DHT by steroidogenic enzymes exerting 3β- and 17β-hydroxysteroid dehydrogenase (HSD) activities [10,11]. In CRPC cells, increased SRD5A1 expression further enhances conversion of androstenedione into DHT through the 5α-androstanedione pathway [12,13]. Alternatively, intratumoral de novo androgen formation from cholesterol has been proposed based on the detection of all steroidogenic enzymes essential for DHT biosynthesis in PC cells [14,15]. To the best of our knowledge, only one study detected de novo steroid production in PC cells [16].

All pathways contributing to the intratumoral androgen pool are potential contributors to CRPC development. Unravelling their differential contributions is instrumental to understanding the impact of current hormonal therapies in CRPC patients as well as for the design of novel anti-hormonal treatment strategies. Here we performed a comprehensive, quantitative analysis of steroidogenic activities within PC cell models and CRPC patient tissue samples using mass spectrometry-based steroid flux analysis.

2. Methods

2.1. Cell lines

The PC cell lines VCaP, PC346C, LNCaP, C4–2B and PC-3 were cultured in T75 flasks (Corning, Sigma Aldrich, Poole, United Kingdom) according to previously described protocols in steroid-containing medium (Gibco, Thermo Scientific, Landsmeer, The Netherlands)[17,18]. Cells were routinely checked for the presence of mycoplasma. VCaP, PC346C and LNCaP are androgen-dependent cell lines. While VCaP and PC346C harbour wild type AR, LNCaP expresses the T878A AR mutation making the receptor promiscuous to activation by non-androgen steroids. C4–2B is a castration-resistant, bone-metastasizing subclone of LNCaP. A cell line resistant to abiraterone, designated VCaP-ABI, was created by long-term culturing of VCaP cells in 10% dextran-coated charcoal-stripped serum (DCC) in the presence of 1 μM abiraterone (Axon Medchem, Groningen, The Netherlands)[19]. PC-3 is androgen-independent as it fails to express the AR.

Cells were plated in triplicate in 6 well plates at 500.000 cells per well for experiments (Corning). After overnight attachment medium was changed to 2% DCC-treated serum containing 1 μM of steroid hormone (Fig. 1). After 24 h supernatants were transferred to hexamethyldisilazane-treated glass tubes and stored at − 20 °C. Cells were immediately lysed using 1 mL Trireagent (Sigma Aldrich); the lysate was stored at − 20 °C until the isolation of RNA. Experiments were performed in triplicate.

2.2. Tissue slices

Informed consent for the secondary use of surplus tissue was obtained from one hormone-sensitive PC (HSPC) and three CRPC patients requiring a transurethral resection of the prostate (TURP) due to local tumor obstruction. The use of anonymized surplus tissue according to the regulations of the Dutch Central Committee on Research Involving Human Subjects (CCMO) was by the Medical Ethics Committee of the Erasmus MC. All CRPC patients had previously been diagnosed with PC and were treated with ADT for more than 6 months. At the time of TURP, one CRPC patient was on investigational treatment with orteronel, a nonsteroidal 17,20-lyase inhibitor. During the procedure, malignant tissue (visible as a tumor bulge during cystoscopy in all patients) was collected. Fragments were kept on ice until further processing.

Fragments were sliced into 300-micron slices using a Leica VT1200 S microtome (Leica Microsystems B.V., Amsterdam, The Netherlands) and kept in prostate growth medium (PGM) supplemented with steroid-stripped FCS in a 24-wells plate at 37 °C and 20% oxygen. After an overnight washout, medium was refreshed with steroid-stripped PGM

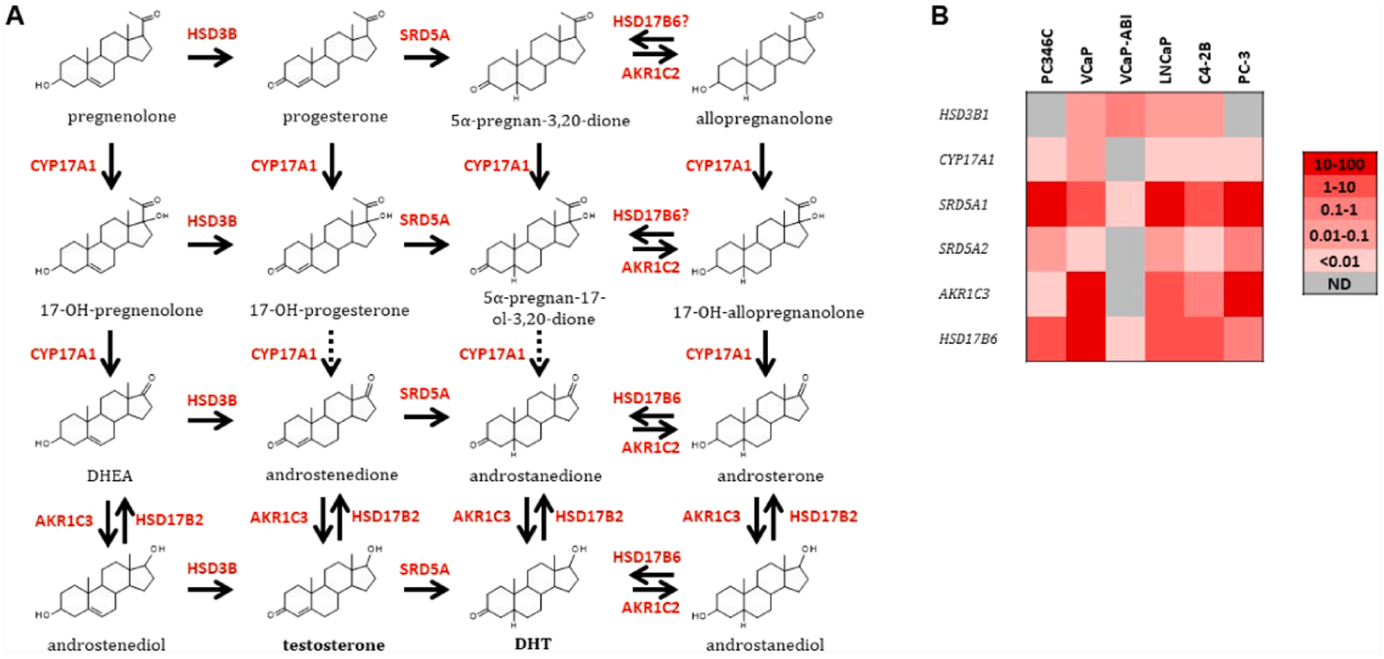

Fig. 1. Androgen biosynthesis. (A) Overview of the classic and alternative androgen biosynthesis pathways. Testosterone and DHT are the active androgens by stimulating the wildtype AR. Gene names of steroidogenic enzymes are depicted in red. Dotted lines represent enzymatic conversion with low efficiency. Abbreviations: DHEA: dehydroepiandrosterone, DHT: 5α-dihydrotestosterone. (B) Heatmap of expression of the most relevant androgen-producing enzymes in 6 tested PC cell lines. Expression is calculated relative to GAPDH.

with substrate added at 1 μmol/L in duplicate. After a 24-hour incubation period, medium was stored at − 20 °C and slices were either stored at − 80 °C or fixed in formalin and subsequently embedded in paraffin for histologic validation of tissue type.

2.3. Liquid chromatography-tandem mass spectrometry

Supernatant steroid concentrations were quantified by liquid chromatography-tandem mass spectrometry (LC-MS/MS) using a Waters Xevo mass spectrometer with Acquity uPLC system and a Waters HSS T3 column (2.1 mm × 50 mm, 1.8 μm, Elstree, United Kingdom). LC-MS/MS used an electrospray ionization source in positive mode with capillary voltage 4.0 kV, a source temperature of 150 °C, and a desolvation temperature of 500 °C. Steroids were extracted from 1 mL of supernatant by liquid-liquid extraction with tert-butyl-methyl-ether (Sigma Aldrich)[20]. After the evaporation of ether, samples were dissolved in 200 μL of 50/50 methanol/water. All steroids were separated using an optimized gradient system consisting of methanol and water with 0.1% formic acid and quantified referring to a linear calibration series with appropriate internal standards. Fifteen steroids from the classic and alternative androgen biosynthesis pathways (Fig. 1A) were identified by matching retention times and two mass transitions in comparison with a deuterated reference compound as previously reported [21]. Multiple reaction monitoring transitions with the least interference from potential co-eluting compound were chosen, however, androstenediol could not be quantified due to incomplete chromatographic separation and interference from testosterone. The lower limit of quantification (LLOQ) was 0.5 ng/mL, as this was the lowest calibration point. At this concentration, for all steroids we observed a gaussian shaped peak with a signal-to-noise ratio greater than 10. Additional quality procedures were employed where the liner correlation coefficient of the calibration line (R^2) was greater than 0.98, and the deviation from the theoretical concentration was $< 20\%$ at the LLOQ and $< 15\%$ at all other calibration points for the experiment to be acceptable. The LC-MS/MS raw data were processed using MassLynx (v4.1, Waters).

2.4. RNA isolation, cDNA synthesis and real-time PCR

RNA was isolated by Trireagent using the manufacturer's protocol. Concentration and purity of RNA was measured using Nanodrop photospectrometer (Thermo Scientific). From the RNA cDNA was synthesized in a 20 μL mix containing 1 μg RNA, 3125 U/μL multiscribe reverse transcriptase, buffer, 5.5 mM $MgCl_2$, 500 μM deoxynucleotides, 2.5 μM random hexamers, oligo(dT) and 0.4 U/μL RNase inhibitor. The mix was placed in a Geneamp PCR amplifier (Thermo Scientific) in a program of 10 min at 25 ºC, 60 min at 37 ºC, 30 min at 48 ºC and 5 min at 95 ºC. cDNA was stored at − 20 ºC.

Real time quantitative PCR of steroidogenic enzymes (*HSD3B1*, *CYP17A1*, *SRD5A1*, *SRD5A2*, *AKR1C3*, *HSD17B6*) and AR target genes (*FKBP5* and *TMPRSS2*) was performed in duplicate using 16 ng of cDNA, PCR mastermix (Thermo Scientific) and Taqman assays on demand (Thermo Scientific). The reaction was done according to standard protocol in an Applied Biosystems PCR 7500 system. Threshold cycles (CT) were calculated with accompanying software and mRNA expression was calculated relative to the average CT of housekeeping gene *GAPDH* [22].

2.5. Statistical analysis

Data analysis was performed using GraphPad Prism (version 9.0.2., San Diego, CA, USA). ANOVA with post hoc Dunnett's test was performed using the average of three independent experiments. mRNA levels were logarithmically transformed before analysis. All analyses were done as two-tailed and $P < 0.05$ was considered statically significant.

3. Results

3.1. Presence of steroidogenic enzymes

Baseline presence of enzymes involved in androgen biosynthesis (Fig. 1A) was tested in the six different PC cell lines (PC346C, VCaP, VCaP-ABI, LNCaP, C4–2B, PC-3) with varying AR expression and dependencies. Confirming previous findings [11] *CYP17A1, HSD3B1* and *SRD5A2* mRNA were low to undetectable in all PC cell lines investigated (Fig. 1B). Enzymes downstream of CYP17A1 were expressed at higher levels, with some variability between the studied cell lines. No obvious relation between steroidogenic enzyme expression and reported androgen dependency of the cells could be observed.

3.2. Steroid flux measurements in cell lines

Following pilot experiments with incubation times varying between 4 and 72 h, we selected an incubation period of 24 h for the determination of steroid production and mRNA expression. Incubation with a 1 μmol/L steroid substrate concentration was chosen to facilitate quantification of at least 0.15% conversion to downstream steroid metabolites.

Across the cell lines, up to five steroid metabolites were detected following incubation with a single steroid substrate. This was the result of a maximum of three enzymatic activities downstream of the employed steroid substrate (Fig. 2, Supplementary Table 1A-B). However, we did not detect CYP17A1 activity, neither 17α-hydroxylase and 17,20-lyase, in any of the cell lines. Using 8 different substrates (pregnenolone, 17α-hydroxypregnenolone, progesterone, 17α-hydroxyprogesterone, 5α-pregnan-3,20-dione, 5α-pregnan-17-ol-3,20-dione, allopregnanolone, 17α-hydroxyallopregnanolone) in 5 parental cell lines no steroid product downstream of CYP17A1 could be detected. Given reports of upregulation of *CYP17A1* expression following ketoconazole or abiraterone [15], steroid flux analysis was also performed in abiraterone-resistant VCaP-ABI cells. Similar to its parental cell line, no CYP17A1 activity could be detected in this model of abiraterone resistance (Fig. 2).

3α-, 3β- and 17β-hydroxysteroid dehydrogenase and 5α-reductase activities were present in all cell lines to varying degrees (Fig. 2). In the androgen-dependent cell lines PC346C, VCaP, VCaP-ABI and LNCaP these enzyme activities facilitated the production of the alternative pathway substrates 5α-pregnanedione and allopregnanolone. Similarly, 17α-hydroxyprogesterone was metabolized to its respective 5α-reduced and 3α,5α-reduced conversion products, which were metabolized interchangeably through 3α-reduction/-oxidation. However, 5α-androstanedione or androsterone were not detected.

Interestingly, there was ample conversion of steroids substrates downstream of CYP17A1. VCaP(-ABI), LNCaP, C4–2B and PC-3 were capable of metabolizing DHEA into androstenedione, whereas PC346C cells lacked HSD3B1 expression and activity. In all cell lines tested, androstenedione was predominantly metabolized to 5α-androstanedione with a much smaller proportion activated to testosterone. Testosterone to DHT conversion could be detected in all PC cell lines with the exception of LNCaP. Similarly, conversion of 5α-androstanedione to DHT could be detected in all cell lines except LNCaP. However, it cannot be ruled out that trace amounts of DHT below the limit of quantification were produced. In general, 17β-reductase activity was low; only in VCaP-ABI and PC346C conversion of 17β-oxidized steroids was higher than the opposite reaction. For PC-3 no 17β-reductase activity could be detected using four different substrates. Overall, the conversion of C19 steroids by 3α-reduction and 3α-oxidation activities was higher compared to that of C21 steroids, upstream of CYP17A1.

3.3. Androgen receptor activation assay

To confirm steroid-induced AR activation, expression of androgen-

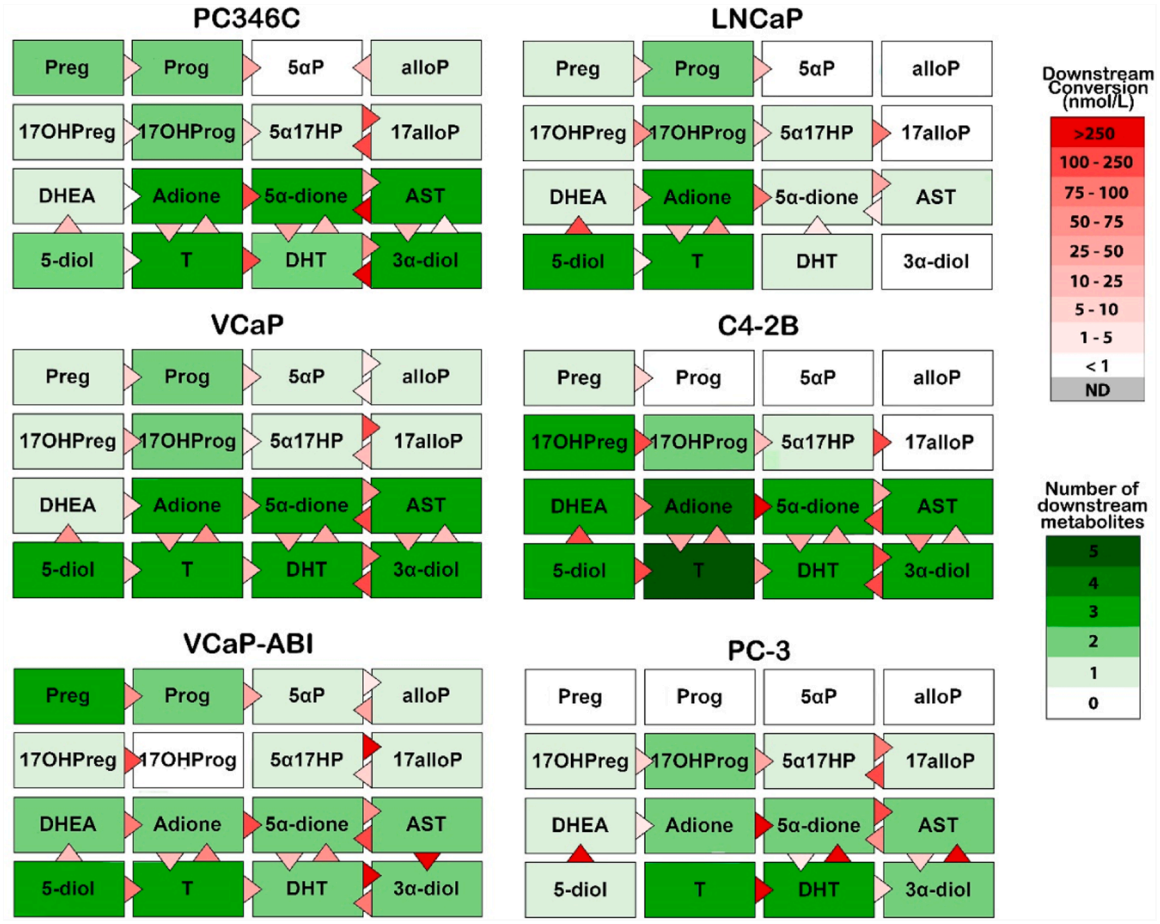

Fig. 2. Steroidogenic capacity in prostate cancer cell lines. All sixteen steroid substrates were incubated at 1 μmol/L in all six cell lines. Concentrations of metabolites converted by steroidogenic enzymes are indicated by color-coded arrows. The number of downstream metabolites is depicted in green for each incubated steroid. No 17-hydroxylase or 17,20-lyase activity was detected across all steroid incubations and cell lines. Abbreviations: 17alloP, 17α-hydroxyallopregnanolone; 17OHPreg, 17α-hydroxypregnenolone; 17OHProg, 17α-hydroxyprogesterone; 3α-diol, 3α-androstanediol, 5α17HP, 5α-pregnan-17-ol-3,20-dione; 5α-dione, 5α-androstanedione; 5αP, 5α-pregnan-3,20-dione; 5-diol, androstenediol; adione, androstenedione; alloP, allopreganolone; AST, androsterone; DHEA, dehydroepiandrosterone; DHT, 5α-dihydrotestosterone, Preg, pregnenolone; prog, progesterone; T, testosterone.

responsive genes was measured. *FKBP5* mRNA was significantly stimulated in both PC346 and VCaP cells by androstenedione, testosterone, 5α-androstanedione, androsterone, DHT and 3α-androstanediol (Fig. 3). DHEA-induced AR activation mirrored the expression and activity of HSD3B1 in the wildtype AR models, being absent in PC346C, low in parental VCaP and high in VCaP-ABI. All 8 CYP17A1 substrates failed to induce *FKBP5* expression in AR wildtype PC346C and VCaP cells. In the AR T878A mutant LNCaP and its CRPC derivative, C4–2B, all incubated steroids significantly increased expression of *FKBP5*, confirming AR pathway activation through receptor ligand promiscuity. It should be noted that, considering the limits of quantification of the LC-MS method, trace quantities of DHT below the detectable limits could have potentially contributed towards AR activation upon incubation with C19-steroids in the LNCaP cells. All steroids except 5α-pregnanedione also stimulated mRNA levels of *FKBP5* in C4–2B, although less potently than LNCaP. As expected, AR-negative PC-3 cells showed no response in *FKBP5* levels following incubation with any of the 16 steroids. Similar results were obtained with the androgen-responsive gene *TMPRSS2* (data not shown).

3.4. Tissue slices of patient samples

In order to validate the findings of the PC cell lines, fresh thin-cut slices from patient HSPC and CRPC tissues were cultured ex vivo. The tissue slices were incubated with key steroid intermediates from both the classic and the alternative androgen biosynthesis pathways. Results are shown in Fig. 4 and Supplemental Table 1C-D. All HSPC and CRPC tissue slices lacked CYP17A1 activity. Conversion of CYP17A1 substrates by 3β-HSD and 3α-HSD was low across all tissue slices with DHEA to androstenedione metabolism limited to less than 0.5%. Conversion of androstenedione to downstream androgens was detected only in the CRPC tissues.

4. Discussion

Here we have performed for the first time a comprehensive screen of classic and alternative androgen biosynthesis pathway activity in prostate cells, with steroid flux analysis quantified by tandem mass spectrometry. Testing across androgen-sensitive and CRPC counterparts of PC cell lines and 4 PC patient tissues showed an absence of any detectable CYP17A1 activity while adrenal androgen precursors were converted into the canonical AR ligands testosterone and DHT. Even in an abiraterone-resistant model and a patient with progressive disease while treated with a 17,20-lyase inhibitor no counterregulatory activation of 17-hydroxylase or 17,20-lyase activities could be detected. These findings support that PC cells are capable to convert adrenal androgen precursors to activate androgens while refuting intratumoral de novo steroid biosynthesis.

Tissue testosterone levels in PC are equal before and after castration [23], implying significant intratumoral androgen production or

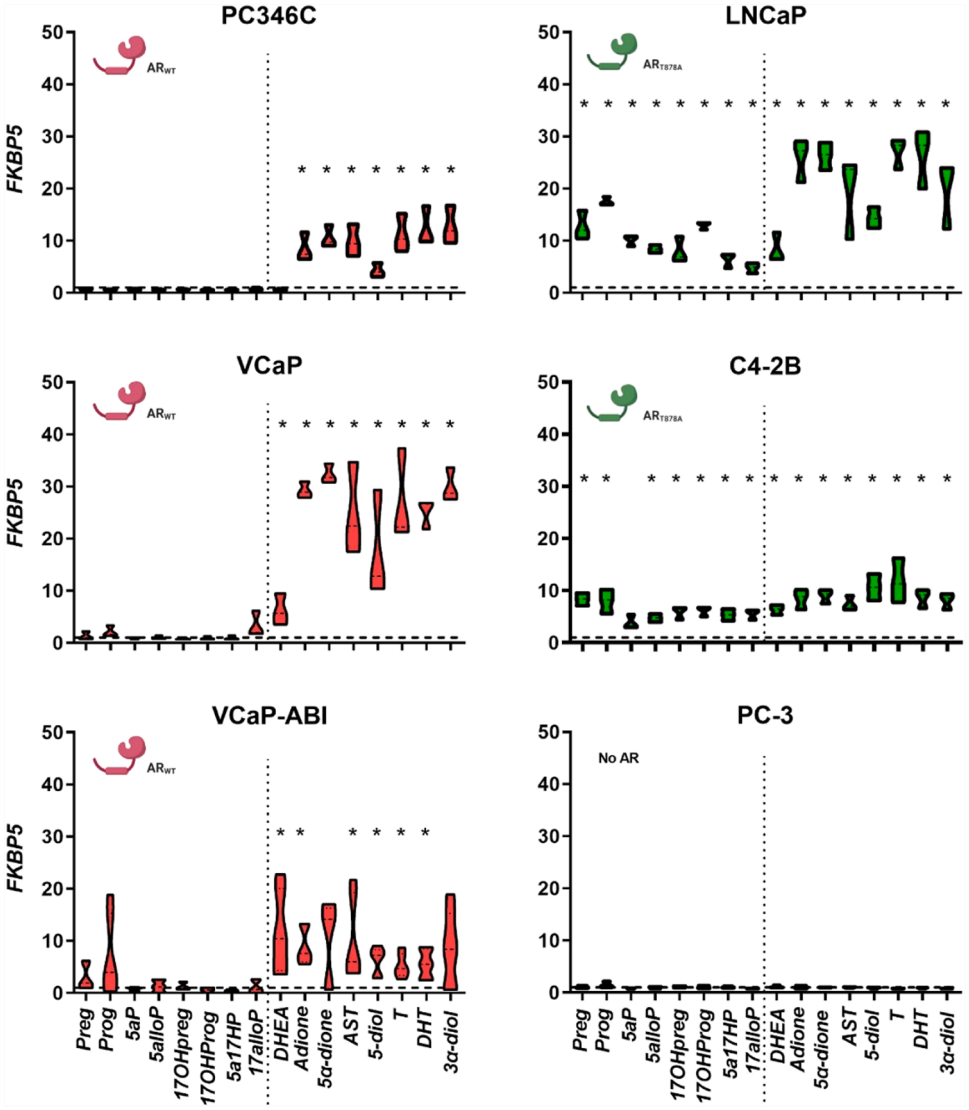

Fig. 3. *FKBP5* expression following incubation of androgens and their precursors. Violin plots of the expression of the androgen-responsive gene *FKBP5*, relative to a vehicle control indicated by horizontal line. The vertical dotted line divides steroids upstream (left) and downstream (right) of 17,20-lyase activity by CYP17A1. PC346C, VCaP and VCaP-ABI possess a wildtype AR, indicated in red. LNCaP and C4–2B express the promiscuous T878A-mutant AR and are depicted in green. PC-3 cells do not express the AR, rendering them androgen-unresponsive.

accumulation. The stimulation of 17β-HSD type 5 (AKR1C3) expression and activity following androgen deprivation could well facilitate increased turnover of androstenedione into testosterone or 5α-androstanedione into DHT, respectively [11,22]. This is supported by our demonstration of steroid fluxes in PC cell lines and tissue slices.

Androstenedione is present at approximately 5 nmol/L circulating concentration in men under ADT, similar to that of DHEA [24]. Moreover, a micromolar depot of DHEAS is retained in these men, offering the potential for intratumoral conversion in relevant androgen concentrations through steroid sulfatase and 3β-HSD [25]. The hypothesis that circulating adrenal androgen precursors are the predominant source of intratumoral androgens is supported by clinical evidence that these steroids can serve as biomarkers for prediction of response to abiraterone [26] and overall prognosis [27].

De novo steroid biosynthesis has previously been proposed based on observations of increased expression of *CYP11A1, CYP17A1*, and *HSD3B1* in advanced PC [12,16,28,29]. Through the combined activities of these enzymes PC cells could potentially be able to produce androstenedione from cholesterol. Since this hypothesis was first postulated, only one study has shown non-quantitative evidence of de novo androgen biosynthesis[16]. Whether this would lead to relevant androgen levels within prostate tumor tissue was unknown to date. Here we show a lack of CYP17A1 activity in all PC cell lines and fresh patient samples studied, which effectively prevents the generation of androgens from cholesterol and also precludes DHT biosynthesis via the alternative pathway. As our current LC/MS-MS setup quantifies as low as 0.15% conversion rates, these results present strong evidence against the presence of relevant de novo androgen biosynthesis in PC cells. This is supported by the lack of stimulation of androgen-responsive genes by any steroids upstream of CYP17A1 in cells with wildtype AR. Also in a resistant model to CYP17A1 inhibition no activity of this enzyme was observed, confirming our previous in vivo descriptions [15]. Despite the presence of low but detectable levels of *CYP17A1* mRNA no enzyme activity could be quantified in six human PC models employing eight substrates and two different read-outs of steroid conversion by LC-MS/MS and AR activation through analysis of AR target gene expression.

Recently, 11-oxygenated androgens were described as relevant AR activators in PC cells at physiological concentrations [30]. In castrate men, 11-ketotestosterone constitutes the most prevalent androgen in

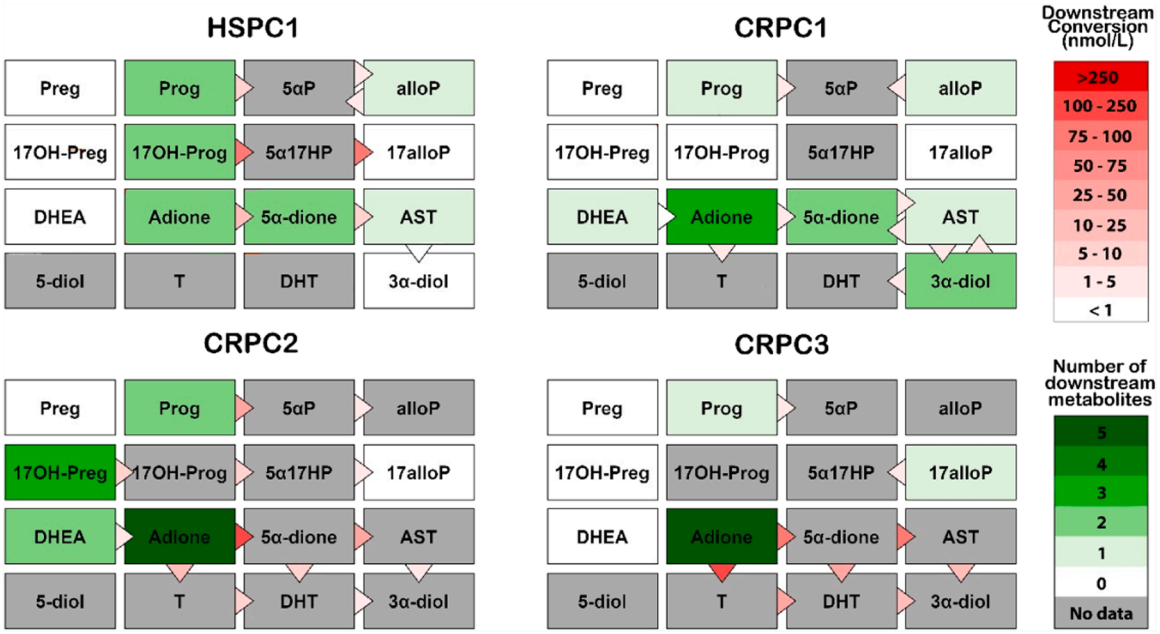

Fig. 4. Steroidogenic capacity in PC patient tissue slices. Steroid substrates were incubated at 1 μmol/L in one hormone-sensitive prostate cancer (HSPC) and three CRPC patient tissue slice cultures. Concentrations of metabolites converted by steroidogenic enzymes are indicated by color-coded arrows. The number of downstream metabolites is depicted in green for each incubated steroid. Steroidogenic capacity was limited upstream of CYP17A1, while more potent metabolism was observed following androstenedione incubation.

circulation [31]. The lack of CYP17A1 activity in (CR)PC cells precludes de novo androgen synthesis of both classic and 11-oxygenated androgens as they depend on generation of the androgen precursor DHEA via CYP17A1 activity. As CYP11B1 and CYP11B2 are expressed exclusively in the adrenal gland, circulating DHEA and androstenedione likely do not fuel intratumoral production of bioactive 11-oxygenated androgens. However, given the expression and activity of the *AKR1C3*, *SRD5A1* and *HSD11B2* in PC cells [31], AR activation could potentially be achieved by intratumoral conversion of 11β-hydroxyandrostenedione into 11-ketotestosterone or even 11-ketoDHT [32–34].

This study confirms the relevance of AR genotype in PC cells and reveals that intracellular steroid hormone metabolism can exert differential effects in cell lines with varying AR status. Wildtype AR has an EC50 of 0.22 nmol/L for Test and 0.29 nmol/L for DHT [35]. Consequently, intracellular conversion of all steroids downstream of CYP17A1 induced AR activation in PC346C, VCaP and VCAP-ABI. The AR is mutated in up to 21% of metastatic PC [36] and several mutations are known to confer promiscuity towards other steroidal ligands, such as progesterone and estradiol [37,38]. Our results generated following incubation of supraphysiological steroid substrate concentrations reveal AR activation not only by pregnenolone and progesterone, but also by their 17-hydroxylated and 5α-reduced metabolites. As these enzymatic reactions are irreversible, the activation of AR is caused by these steroid metabolites or their further downstream molecules. Consequently, accumulation of C21 steroids upstream of 17,20-lyase, for instance following abiraterone treatment, could have potential effects on intratumoral AR activation in patients [39].

We replicated previous findings of a preferred androstenedione to 5α-androstanedione pathway instead of testosterone formation in PC cells [40]. For all cell lines tested, 5α-reduction of androstenedione exceeded conversion by 17β-HSD. Interestingly, in LNCaP cells, conversion of androstenedione through either 5α-androstanedione or testosterone did not lead to detectable DHT formation, possibly through increased conversion to 17β-oxidized steroids or through conjugation by UGT-family enzymes. While other studies have shown that glucuronidation can modulate exposure to bioactive androgens [41], these enzymatic conversions are irreversible and do not yield bioactive steroids. As our methodology only allowed for the quantification of the unconjugated steroid species, we cannot exclude the possibility that small quantities of DHT were produced but were metabolized before they could reach quantifiable levels. In an in vivo system with a constant influx of precursor steroids, this could potentially give rise to a low steady state DHT concentration.

In the PC patient tissues, steroid conversions were broadly comparable to those in PC cell lines. As in PC cell lines, Testosterone, DHT and 5α-androstanedione were detected downstream of androstenedione. Again, the 5α-androstanedione pathway seemed to be favored, although one patient sample also displayed comparable testosterone biosynthesis. The quantity of conversion product was generally more limited than in the cell lines experiments, especially for the various 3α,5α-reduced metabolites. This may in part be a limitation of the experimental setup, and could be attributable to viability in tissue medium, or the presence of stromal cells with limited steroid metabolic activity in the tissue slices,

5. Conclusion

Steroid flux analysis revealed the ability of PC cells to convert adrenal androgens into active androgens but did not support de novo androgen biosynthesis due to a universal lack of CYP17A1 activity. These findings help understand the role of steroid hormones in progression of PC during anti-hormonal drug therapy and focus attention for future drug development on steroidogenic targets downstream of CYP17A1.

Ethics Approval

The use of anonymized surplus tissue according to the regulations of the Dutch Central Committee on Research Involving Human Subjects (CCMO) was granted by the Medical Ethics Committee of the Erasmus MC.

Funding

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CRediT authorship contribution statement

Gido Snaterse: Formal analysis, investigation, visualization, writing: original draft, writing - review & editing. **Angela Taylor:** Formal analysis, Validation, Investigation, Methodology, Writing – review & editing. **Matthijs Moll:** Formal analysis, Investigation, Methodology, Writing – review & editing. **Donna O'Neill:** Investigation, Methodology, Writing – review & editing. **Wilma Teubel**: Resources, Investigation, Methodology, Writing – review & editing. **Wytske van Weerden**: Conceptualization, Funding acquisition, Project administration, Writing – review & editing. **Wiebke Arlt**: Conceptualization, Supervision, Funding acquisition, Methodology, Project administration, Writing – review & editing. **Johannes Hofland:** Conceptualization, Formal analysis, Supervision, Funding acquisition, Investigation, Visualization, Methodology, Writing – original draft, Project administration, Writing – review & editing.

Declaration of Competing Interest

None of the authors reported a conflict of interest.

Data Availability

The data generated in this study are available upon request from the corresponding author.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jsbmb.2023.106446.

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