Steroid metabolome analysis reveals that prostate cancer has potent 5α-reductase, 3α- and 17β-hydroxysteroid dehydrogenase activities, but lacks 17-hydroxylase/17,20-lyase

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**Introduction**

Prostate cancer (PC) is dependent on androgen receptor (AR) activation by its canonical ligands testosterone and 5α-dihydrotestosterone (DHT). Intratumoural androgens persisting after castration give rise to castration-resistant PC (CRPC). These intraprostatic androgens levels are hypothesised to result from either adrenal androgen conversion or intratumoural de novo DHT synthesis through the classic or alternative pathways. Quantifying the steroid fluxes responsible for CRPC development can help optimize current endocrine treatment strategies.

Five common PC cell lines were incubated with 1 µM of 16 steroid intermediates of the classic and alternative metabolite concentrations with liquid chromatography/tandem mass spectrometry (LC-MS/MS). Expression of steroidogenic enzymes and AR-responsive genes was estimated by quantitative PCR.

**Methods**

Fig. 1: Steroidogenic pathways

Common steroid precursor cholesterol is converted into pregnenolone by side-chain cleavage. Multiple steroidogenic enzymes convert pregnenolone into the active androgen dihydrotestosterone (DHT) through either the classic pathway (left) or the alternative pathway (right blue).

**Results**

Fig. 2: Steroid metabolome analysis in prostate cancer cells

Four prostate cancer cell lines were incubated with 1 µM of steroid hormones. Supernatant levels of incubated steroids (red) and their metabolites (blue) were measured through LC-MS/MS. No endogenous pregnenolone production or CYP17 activity was detected. Flux into the alternative pathway occurred from both pregnenolone as well as 17OH—progesterone. Downstream of CYP17 there was potent conversion of androgen (metabolite) in all cell lines tested. CA-2B, a bone-metabasing/castration-resistant clone of LNCaP, showed the highest steroidogenic potential.

**Conclusions**

- First quantitative steroid metabolome of PC cells.
- No evidence supporting intratumoural de novo steroidogenesis or CYP17 activity.
- Precursor C21 steriods divert towards the alternative pathway. The presence of these hormones might suggest a role for activity or further metabolism of alternative pathway steroids in PC evolution.
- Adrenal androgens can effectively be converted into DHT in PC.

**Fig. 3: Basal levels of steroidogenic enzymes**

mRNA expression in prostate cancer cell lines, measured by QPCR. Enzymes for de novo steroidogenesis were expressed at low to absent levels, whereas SRD5A and HSD17B were differentially expressed at higher levels. This reflects the steroidogenic activities observed in the metabolome analysis (Fig. 2).

**Fig. 4: AR activation by steroid hormones**

mRNA-expression of AR-responsive genes TMPRSS2 and FBKPS in prostate cancer cell lines after 24H incubation with 16 steroid hormones (Fig. 1). Values are measured by QPCR and calculated relative to vehicle. Steroids upstream of CYP17A1 only influenced expression in LNCaP and CA-2B cells, that harbour the AR T877A mutation. Steroids downstream of CYP17A1 trigger AR transactivation in all AR-positive cell lines. This reflects the steroidogenic activities observed in the metabolome analysis (Fig. 2).

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