Testosterone metabolites inhibit proliferation of castration- and therapy-resistant prostate cancer

SUPPLEMENTARY MATERIALS



Supplementary Figure 1: Steroidogenesis in prostate cancer: Androgen receptor (AR) affinity peaks in DHT by 5 α -reductase conversion of testosterone. 3 β -adiol or 3 α -adiol (3 β -androstanediol, 3 α -androstanediol) are metabolites of 5 α -DHT, converted by AKR1C1 (3 β -adiol) or AKR1C2 (3 α -adiol) with reduced AR-affinity but increased estrogen receptor affinity. Modified from Knudsen and Penning [9].

Α	ETOH	1μM 3-A	1μM 3-Β	10nM R1881	10nM T	10nM DHT	VCaPrev	D	ETOH	1μM 3-A	1µМ 3-В	10nM R1881	10nM T	10nM DHT	LNCAP
37 kDa	1	1.380	1.191	1.083	1.001	0.835	AKR1C1 relative density	37 kDa	1	1.385	0.888	0.843	3.413	3.414	AKR1C1 relative density
37 kDa		0.673	0.656	0.743	0.583	0.460	AKR1C2 relative density	37 kDa	1	1.836	1.497	1.163	2.127	2.398	AKR1C2 relative density
45 kDa	1	1.143	0.927	0.920	0.692	0.622	AKR1C3 relative density	45 kDa	1	0.565	0.878	0.801	2.081	2.556	AKR1C3 relative density
59 kDa							α-tubulin	59 kDa				_	_	_	α-tubulin
В	ETOH	1µM 3-A	1μM 3-Β	10nM R1881	10nM T	10nM DHT	VCaP	Ε	ЕТОН	1µM 3-A	1µМ 3-В	10nM R1881	10nM T	10nM DHT	_{hiP} LNCaP
37 kDa	1	1.716	1.567	1.502	1.191	1.063	AKR1C1 relative density	37 kDa	1	1.347	1.099	1.904	0.766	0.585	AKR1C1 relative density
37 kDa		1.606	 1.787	1.980	1.121	1.048	AKR1C2 relative density	37 kDa	1	1.410	1.066	1.253	0.219	0.144	AKR1C2 relative density
45 kDa	1	3.944	 3.808	0.031	3.717	 3.273	AKR1C3 relative density	45 kDa	1	1.454	1.265	1.032	1.416	1.215	AKR1C3 relative density
59 kDa	_	_	_	_		-	α-tubulin	59 kDa	_				-	-	α-tubulin
С	ETOH	1µM 3-A	1µM 3-B	10nM R1881	10nM T	10nM DHT	VCaP ^{AA}	F	ETOH	1µM 3-A	1µМ 3-В	10nM R1881	10nM T	10nM DHT	hip LNCaPAA
37 kDa	1	0.901	0.819	0.662	0.737	0.697	AKR1C1 relative density	37 kDa	1	2.137	1.100	1.248	4.113	7.158	AKR1C1 relative density
37 kDa	1	0.663	0.646	0.704	0.822	0.840	AKR1C2 relative density	37 kDa	1	2.080	0.631	0.756	0.113	0.131	AKR1C2 relative density
45 kDa	1	 1.151		 1.324	 1.438	1.306	AKR1C3 relative density	45 kDa	1	1.261	1.053	0.575	1.009	1.182	AKR1C3 relative density
59 kDa	_			-	_		α-tubulin	59 kDa	-					-	α-tubulin

Supplementary Figure 2: Expression of AKR1C1, AKR1C2, and AKR1C3 in prostate cancer cell lines. In VCaPrev cells, 1 μmol/L 3β-adiol, 10 nmol/L R1881, 10 nmol/L testosterone, and 10 nmol/L 5a-DHT resulted in no marked changes in AKR1C1 and AKR1C3 expression, whereas AKR1C2 expression was markedly reduced (A). In VCaP cells, AKR1C1, AKR1C2, and AKR1C3 expression was upregulated after application of 1 μmol/L 3α-adiol, 1 μmol/L 3β-adiol, 10 nmol/L testosterone, and 10 nmol/L 5a-DHT, with the following exception; R1881 had not effect on AKR1C3 (B). VCaPAA expressed AKR1C1, AKR1C2, and AKR1C3. Treatment with 1 μmol/L 3α-adiol, 1 μmol/L 3β-adiol, 10 nmol/L R1881, 10 nmol/L testosterone, and 10 nmol/L 5a-DHT effects downregulation of AKR1C1 or AKR1C2 expression and upregulation of AKR1C3 (C). Low expression of AKR1C1 and AKR1C2 and moderate expression of AKR1C3 was observed in LNCaP cells. 3α-adiol (1 μmol/L), 3β-adiol (1 μmol/L), and R1181 had various moderate effects on the basal expression of these enzymes, whereas treatment with 10 nmol/L testosterone and 10 nmol/L 3β-adiol, or 10 nmol/L R1881 resulted in no changes in the expression of AKR1C1, AKR1C2, and AKR1C3. In contrast to that in LNCaP cells, treatment with 10 nmol/L testosterone and 10 nmol/L 3β-adiol, or 10 nmol/L R1881 resulted in no changes in the expression of AKR1C1, AKR1C2, and AKR1C2, and AKR1C3 expression (E). In addition, the application of AKR1C1 and AKR1C2 in hiPLNCaP cells. Not all compounds affected AKR1C3 expression (E). In abiraterone acetate-treated hiPLNCaP cells, the most significant effects were from testosterone and 5a-DHT, in terms of AKR1C1 upregulation and AKR1C3 downregulation (F).



Supplementary Figure 3: Androgen stimulation in various cell types. In LNCaP, VCaPAA, hiPLNCaP, and hiPLNCaPAA cells, proliferation, as assessed by BrdU-ELISA, was significantly reduced after treatment with 1–10 nmol/L testosterone (**A**, **E**–**H**). Testosterone resulted in no changes in proliferation for PC-3 cells and moderate effects for BPH-1 cells (**B** and **C**), and sustained proliferation in testosterone-sensitive prostate cancer cells (**D**) (n.s. = not significant, *P < 0.05, **P < 0.005, **P < 0.0005).



Supplementary Figure 4: Effects of 5a-DHT on various prostate cancer cells. Treatment with 1–10 nmol/L 5a-DHT in LNCaP led to a significant reduction in proliferation, as assessed by BrdU-ELISA; 0.1 nmol/L resulted in no reduction in proliferation in LNCaP cells (**A**). In PC-3, BPH-1, and VCaPrev cells, no changes in proliferation were detected (**B–D**). Treatment with 0.1 nmol/L, 1 nmol/L, 5 nmol/L, or 10 nmol/L 5a-DHT significantly reduced proliferation in VCaP and VCaPAA cells (**E** and **F**). Treatment of HiPLNCaPs resulted in similar effects as treatment of basal LNCaP (**G**, **H**) (n.s. = not significant, *P < 0.05, **P < 0.005, **P < 0.005).



Supplementary Figure 5: Effects of the non-metabolisable androgen R1881 on various prostate cancer cells. PCa cells were treated with 0.1 nmol/L, 1 nmol/L, 5 nmol/L, and 10 nmol/L R1881. Proliferation of LNCaP, VCaP, VCaPAA, hiPLNCaP, and hiPLNCaPAA cells, as assessed by BrdU-ELISA, was comparable to that with testosterone and 5a-DHT (n.s. = not significant, *P < 0.05, **P < 0.005, **P < 0.0005).