Targeting DHEA-S Transport and Steroid Sulfatase for More Efficient Androgen Deprivation Therapy

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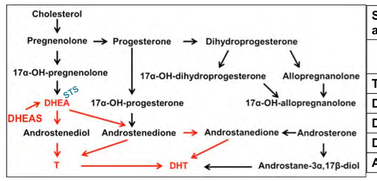
Abstract MP29-11

Background

- Testosterone (T) and dihydrotestosterone (DHT) activate androgen receptor (AR), which is important to prostate cancer.
- Prostate cancer cells are capable of intra-tumoral (intracrine) production of T and DHT.
- Adrenal androgens are the most abundant androgens in humans.
- Dehydroepiandrosterone (DHEA) and DHEA-sulfate (DHEA-S) are the major adrenal androgens in circulation.
- DHEA-S and DHEA may serve as substrates for intracrine T/DHT production.
- Concentrations of DHEA-S and DHEA in circulation are ~3.5 to 10 μM and ~ 10 nM, respectively.
- Steroid sulfatase (STS) hydrolyzes DHEA-S to DHEA.
- DHEA-S remained in μM range after androgen deprivation therapy (ADT) (Snaterse et al., 2017).
- DHEA contributes to 50% prostate DHT in intact men (Labrie et al., 2005).



Serum and prostate tissue levels of androgens (nM)		
	Serum levels	Prostate levels
T	14.6 nM	9.0 nM
DHT	1.6 nM	23.9 nM
DHEA	10.1 nM	23.6 nM
DHEA-S	3.5 μM	42.6 nM
ASD	3.8 nM	-

❖ The present study addressed the capability of prostate cancer cells and tissue to use DHEA-S and DHEA for T/DHT production, delineate signaling pathways activated by DHEA-S and DHEA, and determine whether adrenal androgens sustained tumor growth after ADT.

Experimental Procedures

- Expression of STS was examined using qRT-PCR and immunohistochemistry (IHC).
- Metabolism of DHEA-S and DHEA in intact tissue specimens was evaluated *ex vivo* using fresh clinical prostate tissue.
  - Tissue specimens were cut into 1-3 mm^3 pieces.
  - Pieces of tissue were cultured in phenol red-free RPMI 1640 supplemented with 10% charcoal-stripped fetal bovine serum (CS-FBS).
  - DHT or DHEA in culture medium was measured using ELISA.
- Growth of subcutaneous VCaP xenograft *in vivo*
  - Severe combined immunodeficiency (SCID) or nude mice were "humanized" to mimic human circulating T levels
    - Surgical removal of testes
    - T-silastic tubing implant to "humanize"
    - T-silastic tubing removal to castrate
  - DHEA treatment with DHEA-silastic tubing
  - IGF1R inhibitor BMS-754807 was delivered via oral gavage.

Results

Expression of STS in human prostate tissue and human prostate cancer cell lines

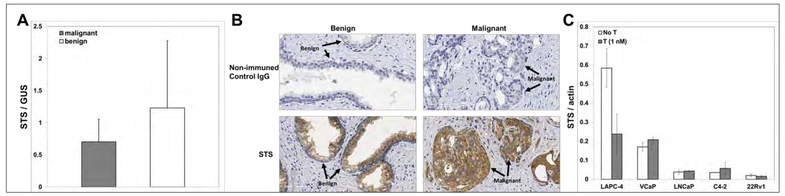
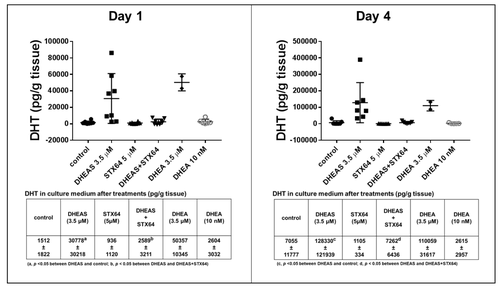


Fig 1. STS expression in matched benign and malignant prostate tissue specimens at (A) mRNA levels (n=20) and (B) at protein levels, and (C) in human prostate cancer cell lines at mRNA levels.

Prostate tissue used DHEA-S and DHEA to produce DHT *ex vivo*



DHT in culture medium after treatments (pg/g tissue)

control	DHEAS (3.5 μM)	STX64 (5μM)	DHEAS + STX64	DHEA (3.5 μM)	DHEA (10 nM)
1512 ± 1822	30778[a] ± 30218	936 ± 1120	2589[b] ± 3211	50357 ± 10345	2604 ± 3032

(a, p <0.05 between DHEAS and control; b, p < 0.05 between DHEAS and DHEAS+STX64)

DHT in culture medium after treatments (pg/g tissue)

control	DHEAS (3.5 μM)	STX64 (5μM)	DHEAS + STX64	DHEA (3.5 μM)	DHEA (10 nM)
7055 ± 11777	128330[c] ± 121939	1105 ± 334	7262[d] ± 6436	110059 ± 31617	2615 ± 2957

(c, p <0.05 between DHEAS and control; d, p < 0.05 between DHEAS and DHEAS+STX64)

Fig 2. DHT production by prostate tissue ex vivo was STS-dependent. STX64, an STS inhibitor. A much higher DHT production on day 4 than day 1 was noted. Low levels of DHT production in control, lower concentrations of DHEA-S and DHEA, and STX64 conditions were noted, although the data points were shown to be close to 0 in the graphs due to the large scale in the Y-axis.

Prostate cancer cell lines used DHEA-S and DHEA to produce DHT

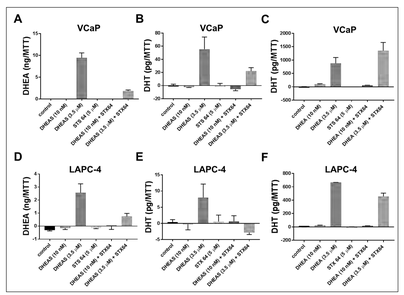


Fig 3. DHT production by prostate cancer cell lines was STS-dependent. A and D showed DHEA production. B and E showed DHT production by cells treated with DHEA-S. C and F showed DHT production by DHEA-treated cells. Androgens in the culture medium were normalized against cell numbers, which was indicated by units of OD570 measured using MTT assay.

DHEA-S activated AR in prostate cancer cell lines

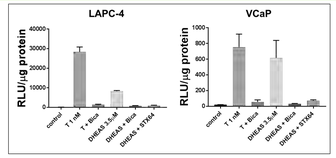


Fig 4. DHEA-S activation of AR was diminished by AR antagonist bicalutamide (Bica) and STX64. AR activity was evaluated using an luciferase reporter-mediated androgen responsive element (ARE) promoter assay.

DHEA-S and DHEA stimulated growth of human prostate cancer cells

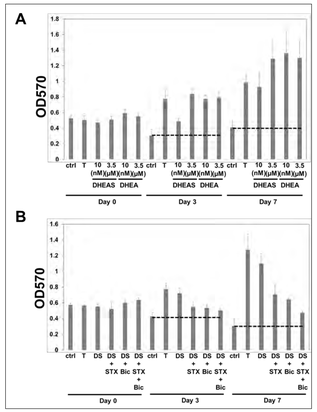


Fig 5. Adrenal androgens stimulated growth of VCaP cells (A). DHEA-S-stimulated growth was diminished by AR antagonist bicalutamide (Bica) and STX64 (B). Growth was assessed using MTT assay, and indicated by units of OD570. DS in panel B was DHEA-S.

Adrenal androgens may have AR-independent functions in prostate cancer cells

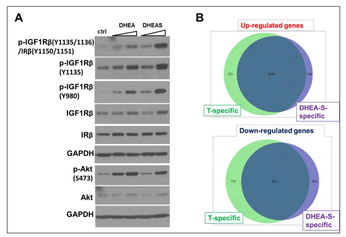
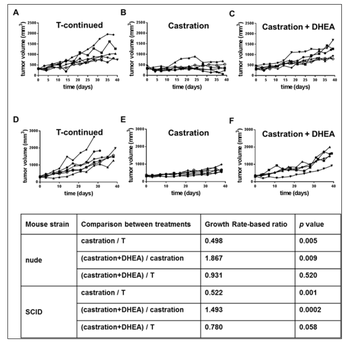


Fig 6. Adrenal androgens activated IGF1R signaling pathway in VCaP cells (A). RNASeq data from the VCaP cells revealed DHEA-S-specific genes that were distinguished from T/DHT-regulated genes (B).

DHEA sustained growth of VCaP xenograft after ADT



Mouse strain	Comparison between treatments	Growth Rate-based ratio	*p* value
nude	castration / T	0.498	0.005
	(castration+DHEA) / castration	1.867	0.009
	(castration+DHEA) / T	0.931	0.520
SCID	castration / T	0.522	0.001
	(castration+DHEA) / castration	1.493	0.0002
	(castration+DHEA) / T	0.780	0.058

Fig 7. DHEA sustained growth of VCaP xenograft after castration in SCID mice (A-C) and nude mice (E-F). Tabled showed ratios of growth rates between treatment groups that were compared.

IGF1R signaling mediated DHEA-stimulated growth of VCaP xenograft after castration

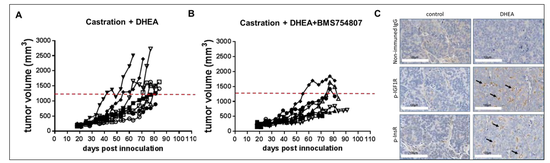


Fig 8. DHEA sustained growth of VCaP xenograft after castration in SCID mice (A). The growth was reduced by IGF1R inhibitor BMS-754807 (B). DHEA increased phosphorylation of IGF1R and insulin receptor (InsR) in VCaP xenograft (C).

Conclusions

- Prostate tissue uses adrenal androgens for DHT production.
- DHEA-S is the most available adrenal androgen for DHT production.
- Adrenal androgens stimulate AR activity and support cell growth.
- Cancer promoting activities of adrenal androgens may be mediated by AR-dependent and AR-independent pathways.
- Identification of potential targets, and therapeutics to block adrenal androgen-stimulated pathways, are necessary to treat prostate cancer.

Support for research

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