Steroid sulfatase stimulates intracellular androgen synthesis and is a therapeutic target for advanced prostate cancer

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Abstract

Background: DHEAS is the most abundant steroid in blood circulation and significant concentrations of DHEAS are present in prostate cancer patients even after ketoconazole or flutamide therapy, suggesting that this may act as a depot for downstream androgen production. Steroid sulfatase (STS) catalyzes the hydrolysis of DHEAS to biologically active DHEA, which is further metabolized to active androgens that bind the androgen receptor (AR) leading to cell proliferation. Currently the role of STS in AR signaling and CRPC is largely unknown. This study determined the role of STS in AR signaling and the potential of targeting STS to overcome castration resistance in prostate cancer.

Methods: Quantitative n-PCR and Western blots were used to detect expression of STS and AR. STS was downregulated using siRNA specific to STS. Stable cell lines overexpressing DHEAS were generated and characterized. RNA-seq was performed on the stable clones. The profiles of the cells were analyzed by GSEA using the Thermo Scientific Vantage UPLC/ABQ system. Testosterone was measured by ELISA in tumor extracts and cell culture media. Eleven potent STS inhibitors (SI) were synthesized and characterized. Prostate cancer cell sensitivity to SI was tested using cell growth assays and clonogenic assays. Efficacy of two SI was tested in vivo in castration-resistant VCaP xenograft tumor models.

Results: STS is overexpressed in CRPC patients and resistant prostate cancer cells including VCaP and 240-MDA cells. Stable STS overexpression in C4-2B and LNCaP cells increases the levels of androstenedione. This resulted in increased cell growth and PSA expression in vitro. Inhibiting STS with siRNA or SI significantly decreases cell growth in androgen-dependent cells. STS overexpression in C4-2B and LNCaP cells promoted resistance to enzalutamide and this could be reversed by silencing STS or STS-significantly reduced AR transcriptional activity, suggesting that inhibition of STS activity by STS downregulates AR signaling. RNAseq analysis demonstrated enrichment of AR and AR-viant signal pathways. Androgen gene sets were reduced by SI-1 and SI-2. SI-1 and SI-2 significantly suppressed the growth of repeated PC3 tumors, reduced intratumoral testisoids, and improved enzalutamide treatment in vitro and in vivo.

Conclusions: These studies suggest that STS drives intracellular androgen synthesis and contributes to prostate cancer proliferation. Targeting STS represents a viable therapeutic strategy to treat CRPC and improve second generation anti-androgen therapy.

Introduction

Intratumoral androgen biosynthesis is well characterized as a mechanism of castration resistant prostate cancer (CRPC). Many enzymes are involved in androgen synthesis, including steroid sulfatase (STS), CYP17A1, another androgenic enzyme, can be upregulated by androgen. However, inhibition of androgen synthesis by androgen deprivation is incomplete, suggesting sustained steroidogenesis in addition to that mediated by STS contributes to resistance. STS is a highly androgenic tissue, and circulating adrenal dehydroepiandrosterone sulfate (DHEAS) to dehydroepiandrosterone (DHEA) may contribute to this sustained androgen production even in the presence of ablation.

STS plays important roles in hormone-responsive malignant growth including breast, endometrial and prostate cancers. In the prostate, the main function is the conversion of adrenal androstenedione sulfatase (17) and DHEAS into estrone and DHEA, respectively, which then feed into the estrogen and androgenic pathways. Numerous STS inhibitors have been tested for potential activity both in animal and clinical settings in breast cancer with some success which demonstrates targeting STS activity is a viable therapeutic approach for cancer. However, while STS inhibitors have been widely investigated in breast cancer, there is little information on the clinical importance of STS in prostate cancer. STS is present in 85% of prostate cancer specimens and its expression is increased in CRPC compared to DHEAS in DHEA. STS is believed to be an alternative source of androsterone which cannot be inhibited by ablation. DHEAS is a major source of androgens for prostate cancer with DHEA and DHEAS is the most abundant steroid in male circulation and is present 400- to 100-fold over DHEA. Therefore, understanding the contribution of STS and DHEA to CRPC progression is of high importance.

STS is highly expressed in CRPC tumors

STS regulates AR signaling

Figure 1. VCaP cells were treated with 10 µM DHT or 25 µM SI-1 or SI-2 with or without 25 µM enzalutamide for 3 days, total cell numbers were counted. B, VCaP cells were transiently transfected with control siRNA or STS siRNA with PSA luciferase plasmid and then treated with 10 µM or with 25 µM enzalutamide for 3 days, cell viability was determined. F. VCaP cells were transiently transfected with STS siRNA or control siRNA and treated with 10 µM or with 25 µM enzalutamide for 3 days, cell viability was determined. p<0.05.