Humanised in vitro co-culture demonstrates CRPC growth depends on adrenal androgens

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Introduction

A rising PSA is one of the hallmarks of castration resistant prostate cancer (CRPC). With PSA being transcribed under an androgen receptor (AR)-specific promoter, this indicates the AR is still active despite low serum testosterone levels. Among other mechanisms of AR-activation, intratumoural androgen synthesis, either via conversion of adrenal androgens or de novo synthesis from cholesterol, is a mechanism of castration resistance. We have previously demonstrated that clinical CRPC samples only expressed markers for conversion of adrenal androgens and not for de novo synthesis1. In this study, we compared growth stimulation of hormone-naïve prostate cancer (PC) and CRPC cell lines by adrenal androgens (conversion) or androgen precursors (de novo synthesis) and tested a novel co-culture model to mimic adrenal stimulation of (CR)PC.

Materials and methods

• VCaP and DuCaP CRPC lines were generated by long-term culturing in androgen depleted medium with or without the antiandrogens bicalutamide or flutamide.
• To test cell growth, VCaP and DuCaP cells and their CRPC sublines were cultured in the presence of androgen precursors (pregnenolone and progesterone) or adrenal androgens (DHEA and androstenedione) at levels found in men.
• Cultures were treated with vehicle, the CYP17A1-inhibitor Abiraterone (0.1 μM) or the anti-androgen MDV3100 (1 μM).
• Cell proliferation was assessed by MIT-assay on day 9 with each experiment performed in triplicate, except for those marked with * (n=1).
• To establish a co-culture system, VCaP cells were cultured with human adrenal (H295R) cells in separate compartments between which only medium could diffuse freely, and treated with vehicle, Abiraterone (1 μM) or DHT (0.1 mM).

Results

1. In hormone naïve and castration resistant VCaP clones, physiological levels of adrenal androgens induced growth while precursors androgens did not

2. Adrenal androgens stimulated DuCaP CRPC proliferation

3. Co-culture of VCaP with H295R cells stimulated VCaP cell growth and is a good model for testing steroid synthesis inhibition in prostate cancer treatment in vitro

Conclusions

In VCaP and DuCaP cells and their CRPC sublines, the substrate for conversion of adrenal androgens induced growth, while the substrate for de novo androgen synthesis did not. These data support our observations that growth of CRPC relies more on conversion of adrenal androgens than on de novo androgen synthesis.

Co-culturing PC cells with human adrenal cells using a 2-compartment system is a relevant model to test CRPC growth stimulation in vitro, demonstrating Abiraterone efficacy. We are currently generating a humanised xenograft model by inoculating PC and H295R cells simultaneously in nude mice to more accurately reflect human CRPC.

References


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