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11-Oxygenated androgen precursors are the preferred substrates for aldo-keto reductase 1C3 (AKR1C3): Implications for castration resistant prostate cancer

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ABSTRACT

The progression of castration resistant prostate cancer (CRPC) is driven by the intratumoral conversion of adrenal androgen precursors to potent androgens. The expression of aldo-keto reductase 1C3 (AKR1C3), which catalyses the reduction of weak androgens to more potent androgens, is significantly increased in CRPC tumours. The oxidation of androgens to their inactive form is catalysed by 17β-hydroxysteroid dehydrogenase type 2 (17βHSD2), but little attention is given to the expression levels of this enzyme. In this study, we show that the 11-oxygenated androgen precursors of adrenal origin are the preferred substrate for AKR1C3. In particular we show that the enzymatic efficiency of AKR1C3 is 8- and 24-fold greater for 11-ketoandrostenedione than for the classic substrates androstenedione and 5α-androstanedione, respectively. Using three independent experimental systems and a computational model we subsequently show that increased ratios of AKR1C3:17βHSD2 significantly favours the flux through the 11-oxygenated androgen pathway as compared to the classical or 5α-androstanedione pathways. Our findings reveal that the flux through the classical and 5α-androstanedione pathways are limited by the low catalytic efficiently of AKR1C3 towards classical androgens combined with the high catalytic efficiency of 17βHSD2, and that the expression of the oxidative enzyme therefore plays a vital role in determining the steady state concentration of active androgens. Using microarray data from prostate tissue we confirm that the AKR1C3:17βHSD2 ratio is significantly increased in patients undergoing androgen deprivation therapy as compared to benign tissue, and further increased in patients with CRPC. Taken together this study therefore demonstrates that the ratio of AKR1C3:17βHSD2 is more important than AKR1C3 expression alone in determining intratumoral androgen levels and that 11-oxygenated androgens may play a bigger role in CRPC than previously anticipated.

1. Introduction

Prostate cancer is a prevalent disease amongst men. The primary treatment of advanced cases of this androgen dependent disease is androgen deprivation therapy (ADT), which is accomplished by physical or chemical castration [1]. While initially effective the cancer invariably re-emerges, and is then termed castration resistant prostate cancer (CRPC). Numerous studies have shown that CRPC remains androgen dependent despite the castrate levels of circulating testosterone and that CRPC is characterised by the reactivation of signalling *via* the androgen receptor (AR) [2]. Indeed, clinical trials with drugs such as abiraterone and enzalutamide, which target the reactivation of the AR-axis, have yielded compelling evidence for the continued role of androgens in CRPC [3–8]. Furthermore, numerous studies have shown that CRPC tumours upregulate the expression of key steroid metabolising enzymes and therefore acquire the ability to convert circulating adrenal androgen precursors to active androgens [9–11]. It should, however, be noted that other mechanisms, such as overexpression of AR, AR mutations and constitutively active AR splice variants, have also been implicated in the development of CRPC [12]. Nonetheless, the inhibition of adrenal derived androgens remains a promising treatment strategy [13].

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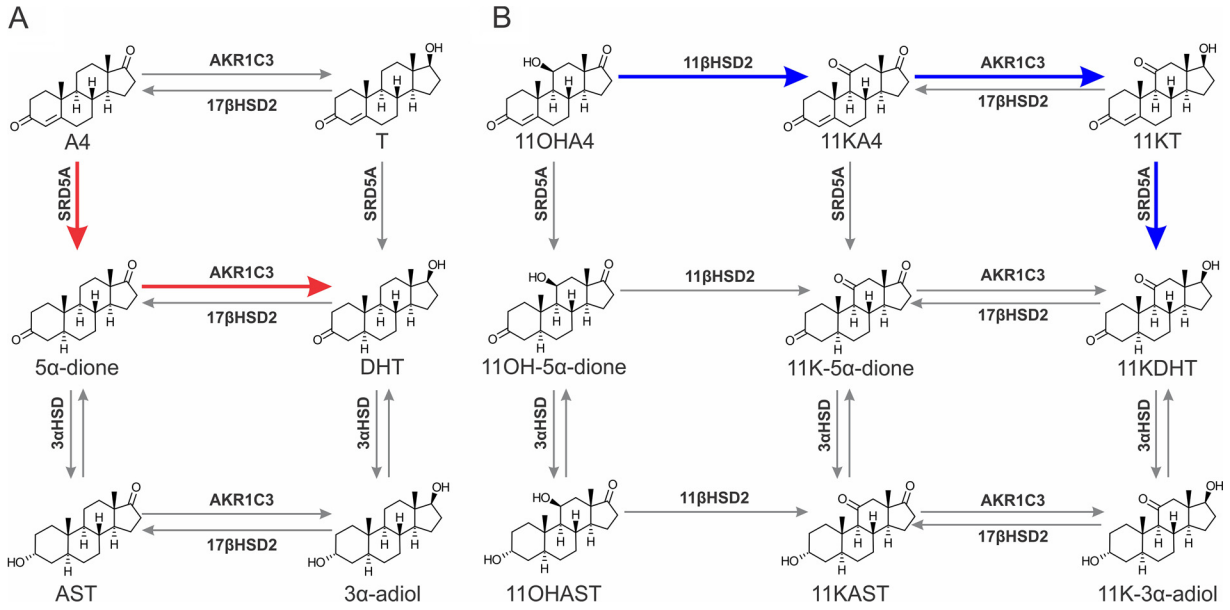

Fig. 1. Schematic of intratumoral androgen activation. The preferred reactions in the alternate 5α-dione (A) and 11OHA4 (B) pathways are shown in bold.

Currently the most recognised pathway pertaining to the intratumoral activation of adrenal androgens is the so-called alternate 5α-dione pathway (Fig. 1A). In this pathway, the adrenal androgen precursors DHEA and androstenedione (A4) are converted to the intermediate, 5α-androstanedione (5α-dione), while bypassing the production of testosterone (T) (Fig. 1A). 5α-Dione is subsequently converted to the most potent androgen, 5α-dihydrotestosterone (DHT), by the enzyme aldo-keto reductase 1C3 (AKR1C3), otherwise known as 17β-hydroxysteroid dehydrogenase type 5 (17βHSD5) [14,15]. Studies by our group have recently shown that the 5α-dione pathway may not be the only pathway contributing to the intratumoral androgen pool in CRPC. We have shown that the abundant adrenal steroid, 11β-hydroxyandrostenedione (11OHA4), is not a dead-end product of adrenal steroidogenesis as previously thought, but rather a precursor to the potent androgens 11-ketotestosterone (11KT) and 11-keto-5α-dihydrotestosterone (11KDHT) (Fig. 1B) [16–18]. 11OHA4 is converted to 11-ketoandrostenedione (11KA4), by 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2). 11KA4 is subsequently converted to 11KT by AKR1C3. 11KT can then be reduced by steroid 5α-reductase type 1 (SRD5A1), yielding 11KDHT, which is equipotent to DHT [16]. Both 11KT and 11KDHT have been shown to induce androgen dependent gene expression, protein expression and cell growth in androgen dependent cell lines and both steroids have been detected in prostate cancer tumours [16,19].

As is evident from above, AKR1C3, plays an important role in both the 5α-dione and 11OHA4 pathways of intratumoral androgen activation. It is therefore not surprising that numerous studies have shown that this is one of the key enzymes that is upregulated during the development of CRPC, presumably to maintain intratumoral androgen levels [10,20–22]. The upregulation of AKR1C3 has also been linked to resistance to both abiraterone and enzalutamide, with combined treatment with the AKR1C3 inhibitor, indomethacin reversing resistance towards both drugs *in vivo* and *in vitro* [23,24]. Furthermore, AKR1C3 expression has been correlated with Gleason score and recurrence status [23]. While AKR1C3 catalyses the 17β-reduction of androgen precursors to their active forms, 17β-hydroxysteroid dehydrogenase (17βHSD2) catalyses the reverse oxidative reactions. The expression levels of 17βHSD2 in CRPC are not often considered with only a few studies showing that the expression of the enzyme remains relatively constant or is downregulated [25]. As AKR1C3 and 17βHSD2 catalyse opposing reactions it is important to consider the expression levels of both enzymes together with their kinetic parameters when considering the flux through intratumoral androgen pathways. We therefore set out to characterise both enzymes towards classical and 11-oxygenated substrates. We subsequently investigated the effect of different AKR1C3:17βHSD2 ratios on the flux through the 5α-dione and 11OHA4 pathways. Our results show that the 11-oxygenated androgen precursors, 11KA4 and 11keto-5α-androstanedione (11K-5α-dione) are significantly better substrate for AKR1C3 than A4 or 5α-dione, and that increased AKR1C3:17βHSD2 ratios have a significantly greater effect on the flux through the 11OHA4 pathway when compared to the 5α-dione pathway. This study therefore adds to the growing body of evidence that 11-oxygenated androgens should no longer be ignored when considering the development and progression of CRPC.

2. Materials and methods

2.1. Cell lines

LNCaP and PC3 cells were purchased from the European Collection of Cell Cultures (ECACC). LNCaP cells were cultured in RPMI-1640 media supplemented with 10% FBS, 1.5 g/L $NaHCO_3$, 2.5 g/L D-(+)-Glucose, 1% HEPES, 1% sodium pyruvate and 1% penicillin-streptomycin, while PC3 cells were cultured in HAMs F12K media supplemented with 10% FCS, 1.5 g/L $NaHCO_3$ and 1% penicillin-streptomycin. HEK293 and VCaP cells were purchased from the American Type Culture Collections (ATCC) and cultured in DMEM media supplemented with 10% FCS, 1.5 g/L $NaHCO_3$, 1% sodium pyruvate and 1% penicillin-streptomycin. C4-2B cells, a generous gift from Professor D Neal (University of Oxford, UK) were cultured in RPMI-1640 media supplemented with 10% FBS, 1.5 g/L $NaHCO_3$, 2.5 g/L D-(+)-Glucose and 1% penicillin-streptomycin. All cell lines were grown at 37 °C in 90% humidity and 5% CO_2.

2.2. Steroids

17β-Dihydroandrosterone (5α-androstane-3α,17β-diol; 3α-adiol), androstanedione (5α-androstane-3,17-dione; 5α-dione), androstenedione (androstene-3,17-dione; A4), androsterone (5α-androstan-3α-ol-17-one; AST), 5α-dihydrotestosterone (5α-androstan-17β-ol-3-one; DHT), drospirenone (17-Hydroxy-6β,7β:15β,16β-dimethylene-3-oxo-17α-pregn-4-ene-21 carboxylic acid, DRSP), dutasteride ((5α,17β)-*N*-