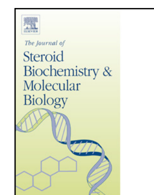
Journal of Steroid Biochemistry & Molecular Biology 155 (2016) 47–55

Contents lists available at ScienceDirect

Journal of Steroid Biochemistry & Molecular Biology

journal homepage: www.elsevier.com/locate/jsbmb



Knockdown of AKR1C3 exposes a potential epigenetic susceptibility in prostate cancer cells

Craig L. Doig[a,*], Sebastiano Battaglia[b], Farhat L. Khanim[c], Christopher M. Bunce[c], Moray J. Campbell[b]

[a] Centre for Endocrinology Diabetes & Metabolism, School of Clinical & Experimental Medicine, University of Birmingham, Edgbaston B15 2TT, UK
[b] Department of Pharmacology and Therapeutics, Roswell Park Cancer Institute, Buffalo, NY 14263, USA
[c] School of Biosciences, University of Birmingham, Edgbaston, B15 2TT, UK

ARTICLE INFO

Article history:
Received 14 March 2015
Received in revised form 24 September 2015
Accepted 26 September 2015
Available online 30 September 2015

Keywords:
Prostate cancer
17B-Hydroxysteroid dehydrogenase
AKR1C3
PPAR
Prostaglandins
Bezafibrate

ABSTRACT

Background: The aldo-keto reductase 1C3 (AKR1C3) has been heavily implicated in the propagation of prostate malignancy. AKR1C3 protein is elevated within prostate cancer tissue, it contributes to the formation of androgens and downstream stimulation of the androgen receptor (AR). Elevated expression of AKR1C3 is also reported in acute myeloid leukemia but the target nuclear receptors have been identified as members of the peroxisome-proliferator activated receptor (PPARs) subfamily. Thus, AKR1C3 cancer biology is likely to be tissue dependent and hormonally linked to the availability of ligands for both the steroidogenic and non-steroidogenic nuclear receptors.
Methods: In the current study we investigated the potential for AKR1C3 to regulate the availability of prostaglandin-derived ligands for PPARg mainly, prostaglandin J_2 (PGJ_2). Using prostate cancer cell lines with stably reduced AKR1C3 levels we examined the impact of AKR1C3 upon proliferation mediated by PPAR ligands.
Results: These studies revealed knockdown of AKR1C3 had no effect upon the sensitivity of androgen receptor independent prostate cancer cells towards PPAR ligands. However, the reduction of levels of AKR1C3 was accompanied by a significantly reduced mRNA expression of a range of HDACs, transcriptional co-regulators, and increased sensitivity towards SAHA, a clinically approved histone deacetylase inhibitor.
Conclusions: These results suggest a hitherto unidentified link between AKR1C3 levels and the epigenetic status in prostate cancer cells. This raises an interesting possibility of a novel rational to target AKR1C3, the utilization of AKRIC3 selective inhibitors in combination with HDAC inhibition as part of novel epigenetic therapies in androgen deprivation therapy recurrent prostate cancer.
© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Nuclear receptors (NRs) regulate multiple gene targets controlling cell growth and differentiation in many self-renewing tissues including hematopoietic and epithelial cells. The ability of NRs to exert these gene regulatory effects is dictated by the supply of ligand availability and epigenetic context (reviewed in [1,2]).

Prostate cancer (CaP) represents an attractive disease with which to target NR signaling in either chemoprevention or chemotherapeutic strategies. Early stage disease is a potential target for chemotherapies that target NRs such as VDR, RARs/RXRs and PPARs [3–5]. Expression of these receptors is sustained in early stage CaP and they are well established as exerting a range of tumor repressive effects. For example, PPARg receptor activation induces anti-proliferative, pro-differentiating gene targets and has subsequently been the target of various clinical trials including a phase II clinical study with Troglitazone [6]. At late stage CaP targeting of the AR by androgen deprivation therapy (ADT) forms the current treatment mainstay for advanced prostate cancer. However, in ADT-R CaP (androgen deprivation therapy—recurrent prostate cancer), is predominantly lethal with limited alternative therapeutic strategies [7].

In late stage and ADT-R CaP aberrant co-repressor actions preclude NR activation, impairing anti-proliferative capacity and further epigenetic mechanisms contribute to this resistance [8] with similar events disrupting AR signaling [9,10] (reviewed in [11]). Specifically, PPARg actions are epigenetically disrupted and can be targeted selectively by using HDAC inhibitor co-treatments

* Corresponding author.
E-mail address: c.l.doig@bham.ac.uk (C.L. Doig).

http://dx.doi.org/10.1016/j.jsbmb.2015.09.037
0960-0760/© 2015 Elsevier Ltd. All rights reserved.

[12,13]. Elevated levels of the co-repressors NCOR1, and to a lesser extent NCOR2/SMRT, correlated with, and functionally drive, the selective insensitivity of PPARa/g receptors towards dietary derived and therapeutic ligands [13,14].

One approach to target NR signaling in CaP is to target the enzymes that regulate ligand availability. Aldoketoreductase 1C3 (AKR1C3) is a multifunctional enzyme and acts as a type 2 3-alpha-hydroxysteroid dehydrogenase or 17-beta-HSD type 5. A key regulator of steroidal metabolism, it is implicated in cancer progression. For example, silencing of AKR1C3 has been shown to inhibit cervical cancer metastasis [15]. In CaP, AKR1C3 actions are implicated in the generation of androgens [16–19], AR activation and has been investigated as a potential biomarker for CaP progression [20,21]. However, AKR1C3 is able to convert various different substrates and reflecting this, it has been implicated in the altered metabolism of chemotherapeutics propagating cancer cells resistance [22]. Perhaps reflecting this promiscuity over substrate choice, AKR1C3 is often overexpressed in prostate cancer tissues and prostate cancer cell lines [23]. Furthermore its expression is elevated in cell lines with either absent or low levels of AR [24] suggesting that substrates may include those independent of androgen signaling and 5α-dihydrotestosterone may not be the only product of AKR1C3 [25,26].

Previous publications have considered the regulatory actions of AKR1C3 on alternative substrates, including the arachidonate-derived prostaglandins that act as *de novo* ligands for PPARg [27]. The expression level of AKR1C3 has been examined in ADT-R CaP tissue and cells lines, including PC-3 and DU 145 cells, and been shown to be elevated compared to less aggressive counterparts and directly proportional to the 11b-PGF_2 levels [28]. We and others have examined the ability of AKR1C3 to convert prostaglandin D_2 (PGD_2), into 9-alpha, 11beta-prostaglandin F_2 [28,29] (a ligand for the FP receptor, which is a driver of cell proliferation) preventing the alternative spontaneous and non-enzymatic generation of the potent PPARg ligand PGJ_2 [30,31]. Additionally, the PPARg mediated protective action of AKR1C3 have been investigated by exploiting 6-Medroxyprogesterone acetate (MPA) an inhibitor of AKR1C3 [32]. In leukemic systems a synergistic effect on cell death occurs with the combination of MPA with PPARg ligand bezafibrate [33]. Combinatorial treatments of MPA and PGD_2 have corroborated this in other cancer models to induce apoptosis and cell cycle arrest through PPARg driven activation pathways [34], and other studies have echoed this approach in CaP [35].

Therefore, the current study, examined the possibility that an AR-independent mechanism for AKR1C3 was operating in CaP cells and, in particular, we focused on a potential role in ADT-R CaP cells where AR signaling is either diminished or lost. By generating CaP cell lines stably expressing a short hairpin siRNA sequence to AKR1C3 we investigated responses to treatment with the PPARg ligand precursor and AKR1C3 substrate PGD_2.

2. Materials & methods

2.1. Ligands

Suberoylanilide hydroxamic acid (SAHA) (Merck Inc, New Jersey, USA), GW9662 (Sigma–Aldrich) and prostaglandin D_2 (Sigma–Aldrich) were stored in DMSO (Sigma–Aldrich) as 100 mM stocks. Bezafibrate (PPARa/g), 6-medroxyprogesterone acetate (Sigma–Aldrich), and indomethacin (Sigma–Aldrich) were stored as 10 mM stocks in DMSO.

2.2. Cell culture and shRNA knockdown

ShRNA targeting AKR1C3 oligonucleotides containing the short hairpin sequence (Sigma–Aldrich) were annealed and inserted in a pcDNA3.1 vector (Invitrogen). Stably transfected cells were exposed to 100 mg/ml neomycin sulphate (Sigma–Aldrich) as a selection agent. Human prostate cell lines used RWPE-1, LNCaP, PC-3, DU 145 were purchased from American Type Cell Culture (ATCC) Manassas, Virginia USA. RWPE-1 cells were maintained within keratinocyte serum free medium (K-SFM) (Invitrogen GIBCO) used in combination with the recommended supplements of bovine pituitary extract (0.05 mg/ml) (BPE) and human recombinant epidermal growth factor (5ng/ml) (EGF). LNCaP, PC-3 and DU 145 cells were maintained in RPMI 1640 medium (Sigma–Aldrich) containing 10% fetal bovine serum (Invitrogen), 2 mM L-glutamine and containing 100 units/ml penicillin and 100 μg/ml Streptomycin. Cells were kept at 37 °C in 95% air and 5% CO_2. Cells were washed in sterile phosphate buffered saline (PBS) and split using Trypsin-EDTA (Sigma–Aldrich) and seeded into new flasks containing fresh media. All experiments were conducted using cells between passages 15 and 28.

2.3. Primary prostate tumor material

All tumors were collected under IRB approval at Roswell Park Cancer Institute (RPCI), specifically the Genitourinary Disease Site Research Network at RPCI, which assesses applications for non-human subject research under guidance of the Office of Research Subject Protection. All patients at RPCI give written consent to allow tumor material not needed for pathological grading to be considered for non-human subject research. Total mRNA from local tumors and adjacent non-neoplastic tissue from the same patient were extracted from snap frozen radical prostatectomy samples with subsequent frozen section analysis for quality control. The frozen section H&E was evaluated by a board certified pathologist for prostatic adenocarcinoma versus benign tissue. Segments of tissue corresponding to prostatic adenocarcinoma with equal to or greater than 70% neoplastic nuclei are submitted for RNA isolation. RNA processing was done in the Pathology Resource Network facilities with standard operating procedures as described previously [36].

2.4. Proliferation assays

Proliferation (ViaLight HS, LumiTech, Nottingham, U.K.) was measured as described previously [37] and optimized using different seeding densities to ensure exponential proliferation through the course of the experiment. Cancer cell lines (2×10^3 cells/well) and RWPE-1 (4×10^3 cells/well) were plated in 96-well, white-walled plates (Fisher Scientific Ltd., Loughborough, U.K.), dosed with agents to final volume of 100 μl/well and incubated for 96 h, with re-dosing after 48 h. Cells were normalized to vehicle control treated wells performed in each separate assay plate. Each treatment was performed in technical triplicate and in biological triplicate experiments.

2.5. Q-RT-PCR

cDNA was prepared using random primers (Promega) and target genes relative expression quantitated using ABI 7500—Applied Biosystems. Sequences for AKR1C3 FORWARD GGGATCT-CAACGAGACAAACG REVERSE AAAGGACTGGGTCCTCCAAGA PROBE TGGACCCGAACTCCCCCGGTG were designed and validated. 18S VIC-labeled probe was used as an endogenous control (Applied Biosystems). Measurements were carried in triplicate, in triplicate wells for each condition and ddCt fold changes calculated.

2.6. Multi-target micro-fluidic Q-RT-PCR_M

Measurement of targeted multiple gene transcripts was undertaken on custom-designed TaqMan Low Density Array

Download English Version:

https://daneshyari.com/en/article/1991361

Download Persian Version:

https://daneshyari.com/article/1991361

Daneshyari.com