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14-3-3 sigma increases the transcriptional activity of the androgen receptor in the absence of androgens

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Abstract

The androgen receptor (AR) is a ligand-activated transcription factor that regulates numerous target genes, including prostate-specific antigen (PSA). We examined the ability of each member of the 14-3-3 family to modulate transcription of PSA through the AR. Despite significant homology within the 14-3-3 family we observed differences in the ability of each isoform to alter the transcriptional activity of the AR. Significantly, 14-3-3 sigma activated PSA-luciferase reporters not only at castrate levels of androgens, but also in the complete absence of androgens. 14-3-3 sigma also increased expression of the endogenous PSA gene in the absence of androgens. Knockdown of the AR by siRNA oligonucleotides abolished activation of these reporters by 14-3-3 sigma. These findings may have greatest significance in hormone refractory prostate cancer where the AR may be activated in a ligand-independent manner.

Keywords: Prostate cancer; 14-3-3 Sigma; Androgen receptor; Transcriptional activator; LNCaP

1. Introduction

Prostate cancer is the second leading cause of cancer death among American men [1]. Organ-confined disease is treatable by means of radical prostatectomy or radiotherapy, but while initially effective, androgen deprivation therapy for metastatic disease is strictly palliative. The eventual emergence of androgen-independent disease leaves patients with few effective treatment options, none of which extend survival by more than a few months. The molecular mechanism of disease progression remains uncertain (see [2,3] for reviews).

The androgen receptor (AR) is a ligand-activated transcription factor required for androgen regulation of genes such as prostate-specific antigen (PSA). Increased expression of PSA in the sera of men after androgen deprivation therapy is the hallmark for identifying androgen-independent disease. Numerous other androgen-regulated genes also become re-expressed with progression to androgen independence, implying that the AR is active in these tissues [3,4].

Ligand-independent activation of the AR is suggested to be a significant contributor to this progression to androgen-independent disease [3,5]. One

Abbreviations: AR, androgen receptor; GR, glucocorticoid receptor; PSA, prostate-specific antigen.

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proposed mechanism by which the AR is activated in the absence of androgens is by the action of coregulatory proteins stimulated by other signalling pathways [5,6]. For example, interleukin-6 (IL-6) caused ligand-independent activation of the AR through the activity of steroid receptor coactivator 1 (SRC1), the mitogen-activated protein kinase (MAPK) pathway, and signal transducer and activator of transcription 3 (STAT3) [7,8]. Increased expression and/or activity of these proteins has also been correlated with prostate cancer and the development of androgen independence [9–12]. Additionally, the coactivator p300 was recently suggested to activate the expression of PSA independent of the AR in LNCaP cells after long term treatment with IL-6 [13].

The 14-3-3 family of proteins regulate a large number of cellular processes, including apoptosis, response to DNA damage, and mitogenic signalling [14]. Members of this family form homo- and heterodimers, which can then bind to a diverse array of target proteins. Binding of 14-3-3s to target proteins modulates the function of those targets by, for example, altering their subcellular localization or protein interaction partners. This family consists of seven different isoforms that share a high degree of sequence identity and conservation [15]. Despite this conservation, numerous isoform-specific observations have been described, including differences in binding partners, subcellular localization, and structure [16–18]. 14-3-3 eta was previously shown to enhance AR transcriptional activity in the presence of ligand, with no effect seen in the absence of ligand [19]. Unfortunately, the effects of other members of this protein family were not reported. Therefore, we investigated the effect(s) of all seven members of the 14-3-3 family of proteins on the transcriptional activity of the AR in prostate cancer cells. We demonstrated that 14-3-3 sigma specifically increased the transcriptional activity of the AR, particularly in the absence of androgens. To our knowledge, this is the first report to examine the effects of all seven 14-3-3 isoforms in the same system under identical conditions.

2. Materials and methods

2.1. Cell culture

LNCaP cells obtained from Dr. L.W.K. Chung (Emory University School of Medicine, Atlanta, GA) were maintained in RPMI 1640 supplemented with 5% (v/v) fetal bovine serum (FBS) (HyClone, Logan, UT), 100 U/mL penicillin, and 100 μg/μL streptomycin. All chemicals were purchased from Sigma, unless stated otherwise. Androgen stimulation was performed by treatment of cells with the synthetic androgen R1881 (Perkin-Elmer, Woodbridge, Canada).

2.2. Plasmids

The ARR_3-tk-luciferase reporter plasmid has been described previously [20]. PSA (6.1 kb)-luciferase was a gift of Dr. J.-T. Hsieh (University of Southwestern Medical Center, Dallas, TX). The expression vectors for the seven isoforms of 14-3-3 were generously provided by Dr. H. Fu (Emory University School of Medicine, Atlanta, GA) [21]. The control expression vector pDEST26 was generated by removing the *BsrGI* fragment from the 14-3-3 zeta-encoding plasmid, eliminating the entire coding sequence. All plasmids were sequence verified.

2.3. Transfection and luciferase assay

LNCaP cells were plated at 3.0×10^5 cells per well in 6-well plates. After 24 h, transfections were performed in serum-free media using Lipofectin® Reagent (Invitrogen) according to the manufacturer's protocol. Each 14-3-3 expression vector was transfected at 500 ng/well unless indicated otherwise, and each luciferase reporter construct was transfected at 1.0 μg/well. The total amount of transfected DNA was normalized to 3 μg/well by the addition of a promoterless plasmid [7,8]. After 24 h serum-free RPMI 1640 containing R1881 (0.001–10 nM), or an equal amount of vehicle (ethanol), was added to the cells. Cell lysates were harvested after 24 or 48 h incubation using Passive Lysis Buffer (Promega, Nepean, Canada). Luciferase activities were measured on a multiplate luminometer (EG & G Berthold, Germany) using the Dual Luciferase Assay System (Promega). Luciferase activities were normalized to the protein concentration of the lysates [7,22] as determined by Bradford assays [23]. All transfection experiments were performed in triplicate wells and repeated at least three times. The results are presented as fold induction relative to pDest26 alone.

2.4. Real-time PCR

LNCaP cells stably transfected with 14-3-3 sigma or pDest26 were treated for 16 h with 10 nM R1881, or an equal amount of vehicle (ethanol), before harvesting total RNA using Trizol® Reagent according to the manufacturer's protocol. Genomic DNA was degraded using the DNA-*free*™ kit (Ambion) according to the manufacturer's protocol prior to reverse transcription using SuperScript® III enzyme (Invitrogen). Quantitative real-time PCR was performed on an ABI 7900 thermalcycler using