The International Journal of Biochemistry & Cell Biology 45 (2013) 763–772

Contents lists available at SciVerse ScienceDirect

The International Journal of Biochemistry & Cell Biology

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

Targeted disruption of the p160 coactivator interface of androgen receptor (AR) selectively inhibits AR activity in both androgen-dependent and castration-resistant AR-expressing prostate cancer cells

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ARTICLE INFO

Article history:
Received 13 August 2012
Received in revised form
24 November 2012
Accepted 13 December 2012
Available online 25 December 2012

Keywords:
SRC-1
Androgen receptor
Prostate cancer
Peptide
CRPC

ABSTRACT

The evidence that androgen blockade-resistant prostate cancer, termed castration resistant, remains androgen receptor (AR) dependent is compelling. AR is re-activated through multiple mechanisms including expression of constitutively active splice variants that lack hormone binding domains (HBDs). This highlights the need to develop therapies that target regions other than the HBD. Because the p160 coactivators interact most strongly with the amino-terminus of AR, we examined the consequences of disrupting this interaction. We identified two overlapping SRC-1 peptides that interact with AR, but not with progesterone receptor. These peptides reduce AR and AR variant AR-V7 dependent induction of an AR responsive reporter. Using mammalian two hybrid assays, we found that the peptides interrupt the AR/SRC-1, AR/SRC-2 and AR N/C interactions, but not SRC-1/CARM-1 interactions. Consistent with the SRC-1 dependence of induced, but not repressed genes, in LNCaP cells, the peptides inhibited hormone dependent induction of endogenous target genes including PSA and TMPRSS2, but did not block AR dependent repression of UGT2B17 or inhibit vitamin D receptor activity. Simultaneous detection of SRC-1 peptides and PSA by double immunofluorescence in transfected LNCaP cells clearly demonstrated a strong reduction in PSA levels in cells expressing the peptides. The peptides also inhibited the AR dependent expression of PSA in castration resistant C4-2 cells. Moreover they inhibited androgen dependent proliferation of LNCaP cells and proliferation of C4-2 cells in androgen depleted medium without affecting AR negative PC-3 cells. Thus, the p160 coactivator binding site is a novel potential therapeutic target to inhibit AR activity.

1. Introduction

Prostate cancer (PCa), an androgen dependent disease, is the second most common cause of death from cancer in American men (American Cancer Society) (Jemal et al., 2010). Locally advanced and metastatic PCa are treated with some form of androgen blockade. Most tumors respond initially, but recur within two years. Androgens act through the androgen receptor (AR), a hormone activated transcription factor that binds to specific DNA sequences and recruits a series of coactivator complexes to modulate transcription of target genes (Mangelsdorf et al., 1995; Shang et al., 2002). Recurrent tumors, termed castration resistant PCa (CRPC) continue to rely on AR action despite reduced levels of circulating androgens (Agoulnik and Weigel, 2006). Recent studies show that some CRPC respond to abiraterone acetate, an inhibitor of adrenal and intratumoral synthesis of androgens, or to MDV3100, a novel non-steroidal anti-androgen, increasing overall survival by a few months in clinical trials (Potter et al., 1995; Tran et al., 2009).

Several mechanisms have been suggested for reactivation of AR. These include increased expression of AR, local synthesis of

Abbreviations: ANOVA, analysis of variance; AR, androgen receptor; ARDH, AR DNA and hormone-binding domains; ARNTD, AR amino terminus and DNA-binding domain; ATCC, American Type Culture Collection; CARM-1, coactivator associated arginine methyltransferase-1; CRPC, castration resistant prostate cancer; DME, Dulbecco's modified Eagle medium; FBS, fetal bovine serum; ECL, enhanced chemiluminescence; GR, glucocorticoid receptor; GRE, glucocorticoid response element; GST, glutathione S-transferase; HBD, hormone binding domain; luc, luciferase; PCa, prostate cancer; PR, progesterone receptor; Qr, glutamine rich; RLU, relative light units; RPMI, Roswell Park Memorial Institute medium; RT-PCR, reverse transcription-PCR; SGK1, serum and glucocorticoid regulated kinase-1; siRNA, small interfering RNA; SRC-1, steroid receptor coactivator; TRβ2, thyroid hormone receptor isoform β2; VDR, vitamin D receptor.

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http://dx.doi.org/10.1016/j.biocel.2012.12.012

androgens, and changes in cell signaling or coactivator expression leading to AR activation (Agoulnik and Weigel, 2006). In addition, AR variants lacking the hormone binding domain are found in many CRPC and may contribute to resistance to current therapies (Dehm et al., 2008; Hu et al., 2009). This highlights the need for therapies that target other regions of AR or reduce overall expression. A previous study showed that over-expression of the region of AR that is amino terminal of the DNA binding domain was sufficient to inhibit androgen-dependent LNCaP xenograft growth (Quayle et al., 2007). This region contains the primary interaction site for steroid receptor coactivator-1 (SRC-1) and likely for the other related p160 coactivators, SRC-2 and SRC-3. In contrast to other steroid receptors, AR interacts weakly with LXXLL motifs of p160 coactivator proteins and interacts predominantly through a glutamine rich (Qr) region in the C-terminus of the p160 coactivators, which contain three small conserved regions termed A, B, and C (Bevan et al., 1999; Christiaens et al., 2002; Ma et al., 1999) (see Fig. 1). Elevated levels of SRC-1 correlate with markers of more aggressive disease (Agoulnik et al., 2005) and tumors expressing high levels of SRC-2/NCoA2/TIF2 recur more quickly than those with low levels of SRC-2 expression (Agoulnik et al., 2006). Recently, Taylor et al. (2010), using genomic profiling, concluded that SRC-2/NCoA2 is an oncogene in 11% of PCa. Thus, we hypothesized that blocking the p160 interacting interface in AR should block AR activity regardless of receptor form or mode of activation. If this is correct, this surface could be a therapeutic target in CRPC. Previous studies have shown that SRC-1 lacking the LXXLL binding motifs retained the ability to interact with and coactivate AR (Bevan et al., 1999). Thus, we sought to determine whether blocking the amino-terminal coactivator binding site using a peptide derived from SRC-1 would be sufficient to block AR dependent transactivation and AR dependent cell growth without inhibiting the actions of related nuclear receptor family members.

2. Materials and methods

2.1. Reagents

R1881 (methyltrienolone) was purchased from PerkinElmer Life Sciences (Boston, MA), calcitriol ($1,25(OH)_2D_3$) from Solvay DuPhar (Weesp, the Netherlands). R5020 (promegestone) from NEN Life Science Products (Boston, MA) and [^{3}H]thymidine from ICN (Irvine, CA). Serum, Triiodo thyronine (T3) and dexamethasone were purchased from Sigma (St. Louis, MO). AR was detected with mouse monoclonal AR 441 antibody (Nazareth et al., 1999) and PR with mouse monoclonal 1294 antibody (Press et al., 2002), kindly provided by Dr. Dean Edwards, Baylor College of Medicine (Houston, TX). The SRC-1 antibody was obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA), the actin antibody from Chemicon (Temecula, CA), and the PSA antibody from DakoCytomation (Denmark). Bound antibodies were detected using ECL (enhanced chemiluminescence) kits from Amersham (Pharmacia Biotech Inc., Piscataway, NJ). Tissue culture plastic ware was purchased from VWR (Sugarland, TX). All other chemicals were reagent grade.

2.2. Plasmids and plasmid construction

P100: To express P100 as a FLAG tagged peptide, the region corresponding to amino acids 1050–1150 of SRC-1 was PCR amplified using a primer containing a flag sequence and an EcoRI restriction site at the 5′ end and a primer containing a NotI restriction site at the 3′ end. Forward primer: CCCGAATTCGCCACCATGGACTACAAGGACGACGATGACAAGCCTA ACCAGCTTCGACTTC. Reverse primer: GCCGCGGCCGCTATAGTCCAGATGAGGGAGG. After digestion with EcoRI and NotI, the PCR product was cloned into

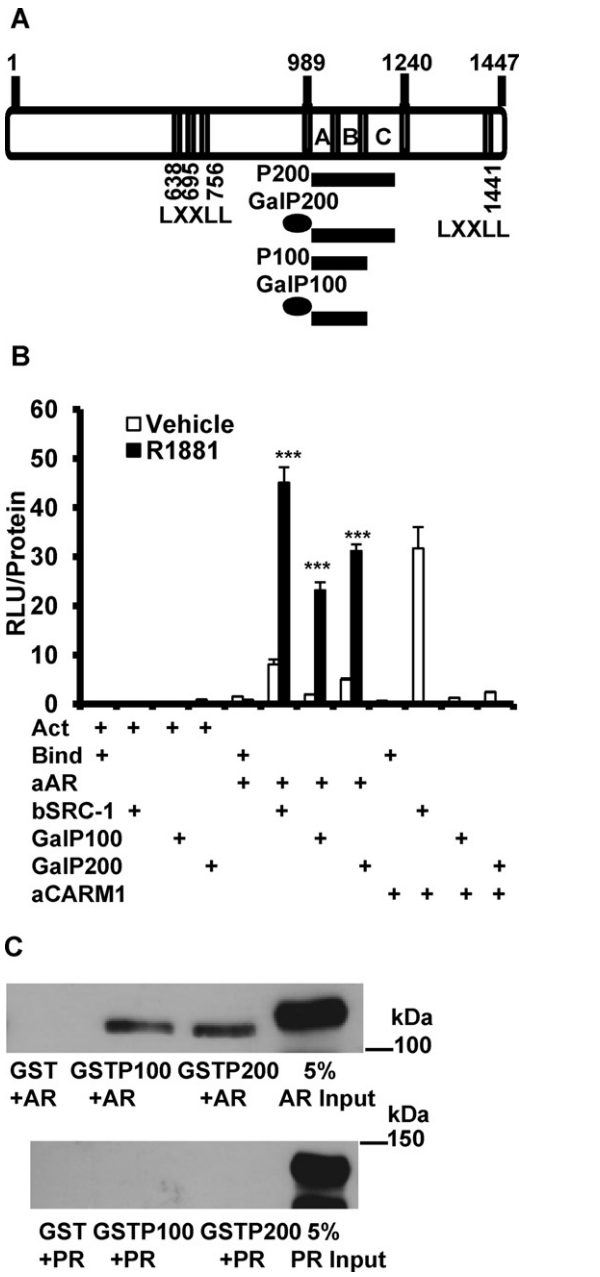

Fig. 1. Design and initial tests of peptides: (A) structure of SRC-1 including the locations of the LXXLL motifs and the location of the Q rich region (989–1240 amino acids) consisting of the A box (1050–1099), the B box (1101–1135) and the C box (1160–1177) box. Also shown are the regions corresponding to the P100 (1050–1150) and P200 (1050–1240) peptides and the corresponding Gal fusion proteins. (B) Interaction of AR and SRC-1 fragments in a mammalian two hybrid assay. HeLa cells were transfected with 250 ng of the Gal4 responsive 17-mer Luc and the indicated combinations of either 100 ng of Act (VP16 control), Act AR, Bind (Gal4DBD control), Gal P100, Gal P200, Bind SRC-1 and Act CARM-1, treated with vehicle or 10 nM R1881, harvested 24 h later, luciferase activity measured and normalized to protein. Each condition was tested in triplicate and average and SEM was calculated. The experiment was done more than three times and a representative experiment is shown. (C) 1 μg of pCR3.1 AR or pCR3.1 PR-B was transfected into COS cells, treated with 10 nM R1881 or 10 nM R5020, cells harvested, and lysed with Promega 1× lysis buffer containing protease inhibitors. Equal amounts of extract were incubated with beads containing GST, GST P100, or GST P200, washed, bound protein eluted and AR and PR detected by western blotting. The experiment was done three times and a representative experiment is shown. ***$p < 0.001$ one way ANOVA with Tukey's multiple comparison test, bind/aAR vs. aAR/bSRC-1, bind/aAR vs. aAR/Gal P100, bind/aAR vs. aAR/GalP200 (in the presence of R1881).