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Corepressor effect on androgen receptor activity varies with the length of the CAG encoded polyglutamine repeat and is dependent on receptor/corepressor ratio in prostate cancer cells

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ABSTRACT

The response of prostate cells to androgens reflects a combination of androgen receptor (AR) transactivation and transrepression, but how these two processes differ mechanistically and influence prostate cancer risk and disease outcome remain elusive. Given recent interest in targeting AR transrepressive processes, a better understanding of AR/corepressor interaction and responses is warranted. Here, we used transactivation and interaction assays with wild-type and mutant ARs, and deletion AR fragments, to dissect the relationship between AR and the corepressor, silencing mediator for retinoic acid and thyroid hormone receptors (SMRT). We additionally tested how these processes are influenced by AR agonist and antagonist ligands, as well as by variation in the polyglutamine tract in the AR amino terminal domain (NTD), which is encoded by a polymorphic CAG repeat in the gene. SMRT was recruited to the AR ligand binding domain by agonist ligand, and as determined by the effect of strategic mutations in activation function 2 (AF-2), requires a precise conformation of that domain. A distinct region of SMRT also mediated interaction with the AR-NTD via the transactivation unit 5 (TAU5; residues 315–538) region. The degree to which SMRT was able to repress AR increased from 17% to 56% as the AR polyglutamine repeat length was increased from 9 to 42 residues, but critically this effect could be abolished by increasing the SMRT:AR molar ratio. These data suggest that the extent to which the CAG encoded polyglutamine repeat influences AR activity represents a balance between corepressor and coactivator occupancy of the same ligand-dependent and independent AR interaction surfaces. Changes in the homeostatic relationship of AR to these molecules, including SMRT, may explain the variable penetrance of the CAG repeat and the loss of AR signaling flexibility in prostate cancer progression.

1. Introduction

The AR is a member of the nuclear receptor superfamily of transcription factors sharing a common evolutionary origin, mode of action and structural architecture, with conserved domains for DNA (DBD) and steroid ligand binding (LBD), and an evolutionarily variable amino-terminal domain (NTD) (Fig. 1A). Ultimately, the capacity of nuclear receptors to function in any given cell depends on recruitment of distinct subsets of cofactors, which control conformation, nuclear-cytoplasmic localization, movement, response element recognition, chromatin remodeling, and engagement or disengagement with basal transcription factors (O'Malley et al., 2008; Wolf et al., 2008). At the transcriptional level, coregulators that activate and inhibit these processes are termed coactivators and corepressors, respectively. For most nuclear receptors, the binding of agonist ligands induces conformational changes and formation of activation function 2 (AF-2), a conserved protein-protein binding surface (Fig. 1A). The AF-2 pocket binds short LxxLL-like peptide motifs in transcriptional cofactors, such as the p160 family of coactivators comprising NCOA1/SRC-1, NCOA2/GRIP1 and NCOA3/AIB1, and acts in concert with activation functions in the NTD of the receptor to mediate overall transcriptional response (Hur et al., 2004; Ozers et al., 2007).

The AR is distinct from other nuclear receptors as the AR AF-2 surface preferentially interacts with the ^{23}FQNLF^{27} peptide of the

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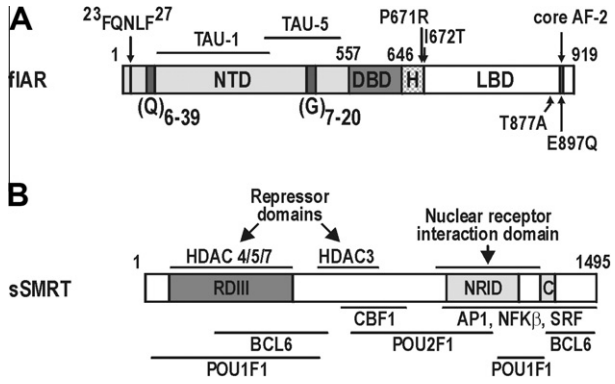

Fig. 1. AR and SMRT constructs used in this study. (A). Schematic representation of the AR showing the N-terminal (NTD), DNA-binding (DBD) and ligand-binding (LBD) domains, and subdomain structures including activation functions (TAU-1, TAU-5, AF-2; Jenster et al., 1992), homopolymeric repeat sequences in the NTD [polyglutamine, $(Q)_n$; polyglycine, $(G)_n$], and the ^{23}FQNLF^{27} peptide required for N/C interactions. The location of point mutations in the AR are shown. Numbers represent the amino-acid residues. (B). Schematic representation of the smaller sSMRT isoform (Chen and Evans, 1995) showing repressor domain III (RDIII), the nuclear receptor interaction domain (NRID), and regions with histone deacetylase (HDAC) activity. "C" denotes the CoRNR box in B. The interaction surfaces for key SMRT cofactors and substrates are depicted below the schematic. Numbers represent amino-acid residues.

AR-NTD in the amino/carboxyl terminal (N/C) interaction, displacing coregulator events to the NTD (He et al., 2006, 2002a). As a consequence, AR activity is almost exclusively determined by two overlapping NTD transcriptional activation units (TAU), TAU-1 and TAU-5 (Jenster et al., 1995; Need et al., 2009) (Fig. 1A). The AR NTD also contains two polymorphic trinucleotide microsatellite repeats, CAG and GGC, which encode respectively polyglutamine (polyQ) and polyglycine (polyG) tracts with a distribution of 6–39 (mean 21) and 7–20 (mean 16) residues in man (Fig. 1A) (Buchanan et al., 2004b).

While the GGC repeat is less variable and usually categorized as being 16 or non-16 repeats, early studies from our group and others showed that the racial distribution of CAG repeats was inversely related to prostate cancer risk (Coetzee and Ross, 1994; Edwards et al., 1999). Subsequent studies confirmed a link between CAG length and risk, age of onset and/or advanced disease at diagnosis (Buchanan et al., 2001a). While *in vitro* and animal studies have related CAG length to receptor activity and prostate size (Albertelli et al., 2008; Buchanan et al., 2004b), more extensive clinical analyses have failed to demonstrate a relationship to disease risk, although did identify a correlation between CAG repeat length and serum testosterone levels (Freedman et al., 2005; Price et al., 2010). We cannot easily account for these inconsistent findings, or indeed for the inconclusive role of CAG repeat length in many other diseases (Rajender et al., 2007), perhaps because the precise effect of CAG and GGC repeat sequences on AR function are unknown.

Steroid receptors have classically been distinguished from those for thyroid hormone, retinoic acid and vitamin D by their relationship with DNA (Laudet, 1997). While unliganded steroid receptors such as AR are thought to be held in an inactive conformation away from DNA by chaperones, those for thyroid hormone and retinoic acid are constitutively bound to DNA in a multi-subunit corepressor complex that actively represses basal gene transcription (Chen and Evans, 1995). In contrast, conventional thought holds that liganded receptors of both classes bind DNA, recruit nuclear receptor coactivators and promote gene transactivation (Biddie et al., 2010; Jepsen et al., 2000; McEwan, 2009; McKenna and O'Malley, 2000).

The molecules at the core of the nuclear receptor corepressor complex are the nuclear receptor corepressor (NCOR1) and silencing mediator for retinoic acid and thyroid hormone receptors (SMRT/NCOR2) (Chen and Evans, 1995; Cunliffe, 2008; Perissi et al., 2010). SMRT and NCOR1 are large scaffold proteins that interact with the hydrophobic AF-2 surface of nuclear receptors using extended LxxLL-like amphipathic alpha helices (LxxI/HIxxxI/L) (the CoRNR boxes) and contain at least three independent repressor domains that alter histone deacetylase (HDAC) activity (Chen and Evans, 1995; Perissi et al., 2010; Stanya and Kao, 2009). Corepressor recruitment inhibits receptor activation, promotes DNA condensation and attenuates binding of other transcription factors (Cunliffe, 2008; Perissi et al., 2010). Thus, receptor occupancy by either repressors or activators is a major determinant of nuclear/steroid receptor transcriptional activity (Glass and Rosenfeld, 2000).

In this study, we sought to better define the relationship between the AR and SMRT, and in particular the role played by AR AF-2 and NTD interaction surfaces, canonical and non-canonical ligands, and variation in the length of the CAG encoded polyglutamine repeat in SMRT-mediated repression. We additionally map SMRT interaction surfaces within the AR NTD, and compare the relative specificity and strength of corepressor recruitment to AF-2 with the AR N/C interaction. Our data provide new information on the dynamic role of corepressors in AR signaling, and have implications for understanding AR antagonists, tissue-specific variation in androgen responses, and how the AR CAG repeat might be variably associated with risk and progression of prostate cancer.

2. Materials and methods

2.1. Plasmid vectors

Expression vector for the full-length smaller isoform of SMRT (pCMX-gal4-h-fSMRT; sSMRT) was provided by Dr. Ron M. Evans (Howard Hughes Medical Institute, Salk Institute for Biological Studies, La Jolla, California). AR-responsive probasin (ARR3-tk-luc), MMTV (MMTV-luc), PSA540 (pGL3-PSA540), GAL-4 targeted pGK1 luciferase reporter constructs, AR expression vectors encoding different polyQ lengths [pcDNA-AR$(CAG)_n$], the constitutive truncated variant (1–709), mammalian two-hybrid AR-NTD (1–538, 1–555), and mammalian two-hybrid wild-type (618–919, 644–919), E897Q and T877A variant AR-LBD constructs have been previously described (Buchanan et al., 2007, 2004b; Need et al., 2009). Mammalian two-hybrid pM-GAL4 and pVP16-AD vectors encoding AR-NTD fragments (encompassing amino acids 1–156, 141–356, 351–426, 427–538, 351–538) were provided by Dr. Ryan A. Irvine (NCCC, USC, Los Angeles, CA). pSG5-GRIP1 was provided by Professor Mike Stallcup (University of Southern California, Los Angeles, California). sSMRT fragments for pM and pVP16 vectors were amplified in PCR reactions using *Pfu* DNA polymerase (Promega, La Jolla, CA) with the following primer pairs: aa1-1495 (5′-CAGAGAATTCATGGAGGCATGGGACGCC-3′, 5′-GTCATCTAGACTTTAGACAGGCAAGGATGCCG-3′); aa1-333 (5′-CAGAGAATTCATGGAGGCATGGGACGCC-3′, 5′-GTCATCTAGATGATGGACCCGCGGATGT-3′); aa298-632 (5′-CAGAGAATTCAGAGCCATCTCCTCAGCCAGCA-3′, 5′-GTCATCTAGAGGTGTCGGGGCAGGTAGTAGGC-3′); aa598-937 (5′-CAGAGAATTCGGCGTGGACCTGTATCGCAG-3′, 5′-GTCATCTAGAGAAGGCATGGCCGGTGTCT-3′); aa890-1266 (5′-CAGAGAATTCACATTCCCACCTGCCACCCACT-3′, 5′-GTCATCTAGACTTGCTTCTTGGACTTGACCAT-3′); a1222-1495 (5′-CAGAGAATTCCTGCTGTACCGGGATGGGGAAC-3′, 5′-GTCATCTAGACTTTAGACAGGCAAGGATGCCG-3′). Products were purified, digested with *EcoR1* and *XbaI* and cloned into the equivalent sites of pM and pVP16 vectors.

2.2. Cell culture, transactivation assays and immunoblot analysis

COS-1 cells (American Type Culture Collection, Rockville, MD) and the PC-3 cell subline PC-3^{AR+}, described previously (Buchanan