Interplay between Two Hormone-Independent Activation Domains in the Androgen Receptor

Leen Callewaert, Nora Van Tilborgh, and Frank Claessens

Abstract

The androgen receptor (AR) plays a key role in prostate cancer development, as well as its treatments, even for the hormone-refractory state. Here, we report that an earlier described lysine-to-arginine mutation at position 179 in AR leads to a more potent AR. We show that two activation domains (Tau-1 and Tau-5) are necessary and sufficient for the full activity of AR and the intrinsic activity of the AR-NTD. Two α-helices surrounding the Lys179 define the core of Tau-1, which can act as an autonomous activation function, independent of p160 coactivators. Furthermore, we show that although the recruitment of p160 coactivators is mediated through Tau-5, this event is attenuated by core Tau-1. This better definition of the mechanisms of action of both Tau-1 and Tau-5 is instrumental for the design of alternative therapeutic strategies against prostate cancer.

Introduction

Prostate cancer is the second leading cause of cancer-related death in men (1). Treatment for prostate cancer relies on eliminating androgen receptor (AR) activation, achieved by reducing circulating androgens to castrate levels and/or blocking ligand binding by AR antagonists. However, in most of the cases, androgen ablation therapy results in prostate cancer relapse (2–4). Recent evidence shows that AR continues to be essential for tumor progression, even in this hormone-refractory state. One possible mechanism whereby tumor cells may adapt to a reduced androgen environment is the development of a hypersensitive AR (5). Thus, understanding the mechanisms that regulate AR function is of critical importance.

AR is a member of the nuclear receptor superfamily, consisting of three functional domains: the NH2-terminal domain (NTD), the central DNA-binding domain (DBD), and the COOH-terminal ligand-binding domain (LBD). Three-dimensional structures are available for the isolated DBD and LBD from both steroid and ligand-binding domain (LBD). Three-dimensional structures are available for the isolated DBD and LBD from both steroid and ligand-binding domain (LBD). These observations leave the coactivation mechanisms of transactivation by AR largely unexplained.

Materials and Methods

Recombinant plasmids and site-directed mutagenesis. The expression vectors pSG5AR [expressing Flag-tagged full-length human AR (hAR)], pSG5ARΔ38-919 (encoding the hAR-DBD-LBD), pSG5SRC1e, and pSG5SRC1eM123 (expressing full-length SRC1e or mutated SRC1e); the vectors SRC1-Qr and AR-NTD (expressing the Q-rich domain of SRC1) and the AR-NTD [fused to the Gal4DBD and the etoposide (VP-16) activation domain] are described elsewhere (9, 11, 15, 20). Expression vectors for the AR-NTD, carrying the mutations of the AF-1 domain, were made by site-directed mutagenesis using the PCR-based method. As template, the expression vector for the full-length AR and its mutants as templates. Insertion of multiple copies of the core Tau-1 domain in the pABGal4 vector was done following by sequencing to determine the number of copies. The same was
done for the Tau-5-DBD constructs containing several copies of core Tau-1. Expression vectors for the AR-Tau-5-DBD, carrying deletions in the Tau-5 domain, were made using the PCR-based method. The same deletions in the full-length AR-NTD-DBD were made by restriction digestion of the Tau-5 deletion mutants with Bsp68l and HindIII, ligated into the AR-NTD-DBD construct, which was digested with the same restriction enzymes. The same procedure was followed for the AR-ΔTau-1 construct. The ARΔTau-5 constructs were made by the PCR-based method. Restriction and modifying enzymes were obtained from MBI Fermentas GmbH (St. Leon-Rot, Germany). The luciferase reporter construct driven by the E1b promoter and containing two copies of the rTAT-GRE was described previously (21). TK minimal promoter-driven reporter construct containing the slp and sc upstream enhancers as well as the pb promoter-driven construct have also been described (22). The reporter construct (Gal4)TATA-Luc and the GST-SRC1-Qr (aa 989-1240) construct was a kind gift from Dr. M.G. Parker (Imperial Cancer Research Fund, London, United Kingdom) and was used for measuring the intrinsic AR-NTD/core Tau-1/Tau-5 activities, for the two-hybrid assays and glutathione S-transferase (GST) pull-down experiments with SRC1-Qr. The reporter plasmid pMMTV-luc was obtained from Dr. P. Chambon (Institut de Génétique et de Biologie Moléculaire et Cellulaire, Illkirch, France). The pCMV-β-Gal vector was obtained from Stratagene (La Jolla, CA).

Transfection assays. All transfections and reporter assays were done in COS-7, Hela, U-2OS, HEK293, CHO-K1, and PC3 cells (American Type Culture Collection, Manassas, VA). For transactivation and two-hybrid assays, the cells were seeded in 96-well culture plates in DMEM or DMEM/F12 (for PC3 cells only; Life Technologies, Gaithersburg, MD) containing 5% dextran-coated, charcoal-stripped (DCC) fetal bovine serum at a density of 30,000 cells/well. After 24 hours of transfection, cells were treated with or without 10^-8 mol/L R1881. Immunostaining was done using M2 anti-Flag antibody (Stratagene) followed by incubation with TRITC-conjugated goat anti-mouse antibody (Sigma-Aldrich, St. Louis, MO). GST pull down. In vitro transcription and translation of AR-NTD-DBD or fragments were done in rabbit reticulocyte lysate in the presence of [35S]methionine in a total volume of 50 μL as described by the manufacturer (Promega). The in vitro translated protein was diluted to 500 μL with binding buffer [20 mmol/L Tris (pH 7.5), 150 mmol/L NaCl, and 0.1% Tween 20]. GST or GST-SRC1-Qr (aa 989-1240) were expressed in the BL21 bacterial strain and bound to glutathione-Sepharose beads (Amersham Pharmacia Biotech). Nonspecific protein-binding sites were blocked by incubation with 2% bovine serum albumin for 1 hour at 4°C. Fifty microliters of each in vitro translated protein was incubated with the beads in 250 μL of binding buffer for 30 minutes at room temperature. Beads were washed thrice with binding buffer. Bound proteins were eluted with 2 × SDS sample buffer. After SDS-PAGE electrophoresis, the gel was fixed in 10% acetic acid/25% isopropanol for 30 minutes, incubated in Amplify NAMP 100 (Amersham Pharmacia Biotech) for another 30 minutes, and dried, and finally, labeled proteins were visualized by exposure to autoradiographic film (Hyperfilm Enhanced Chemiluminescence, Amersham Pharmacia Biotech).

**Immunocytofluorescence staining.** COS-7 cells at 6 × 10^5 were seeded on four-well Labtek II slides (Nalge, Rochester, NY) 24 hours before transfection with 400 ng of AR or AR mutants using Genejuice transfection reagent (Novagen, Madison, WI). After 24 hours of transfection, cells were treated with or without 10^-8 mol/L R1881. Immunostaining was done using M2 anti-Flag antibody (Stratagene) followed by incubation with TRITC-conjugated goat anti-mouse antibody (Sigma-Aldrich, St. Louis, MO).

**Results**

Tilley et al. (19) described a mutation residing in the AR-NTD in a primary prostate cancer biopsy, which consists of the substitution of the Lys^179^ into arginine. We tested several luciferase reporter constructs in transient transfections of COS-7 cells or PC3 cells (Fig. 1A and B, respectively). One construct is driven by the E1b promoter and contains a tandem repeat of the rTAT-GRE. Another construct is driven by the probasin proximal promoter. Two constructs are driven by the thymidine kinase minimal promoter and contain either the slp enhancer or the sc enhancer. In addition, AR activity in PC3 cells was tested on the prostate-specific antigen promoter. We show here that the Lys^179^ mutation into arginine leads to an increased AR activity for all reporter constructs tested, and mutation into glutamine or alanine has the same outcome. The differences are not due to altered expression levels (Fig 1A). The effect of the Lys^179^ mutations on the activity of the full-size receptor prompted us to evaluate its role on both Tau-1 and Tau-5.

**Tau-5 region is necessary for both intrinsic AR-NTD activity and SRC1-Qr recruitment.** The Tau-5 region of AR, between aa 360 and 529, is considered a constitutive activation domain (18). In an attempt to analyze which residues of Tau-5 are important for this intrinsic activity, the activity of the AR-Tau-5-DBD region (aa 360-622) was compared with that of deletion constructs AR-Tau-5D1-DBD, AR-Tau-5D2-DBD (Δaa 360-420), AR-Tau-5D3-DBD (Δaa 421-480), and AR-Tau-5D4-DBD (Δaa 481-529; Fig 2A). Compared with the wild-type AR, the transcription activation capacity of the Tau-5-DBD construct was ~50% or 80% on the rTAT-GRE/E1b-Luc or MMTV-Luc reporters, respectively. For all three truncations of Tau-5, there is a decreased activity. To test the ability of Tau-5 for its interaction with SRC1-Qr, we did a mammalian two-hybrid assay (Fig 2B). A clear interaction with SRC1-Qr is seen for both the AR-NTD-DBD and the Tau-5-DBD constructs. However, deleting any part of Tau-5 leads to an impaired interaction.

We repeated this assay after transfecting the deletions within Tau-5 to the AR-NTD-DBD (Fig. 2C). From those experiments, we can conclude that, indeed, the full Tau-5 region is necessary for the transactivation properties of the AR-NTD (Fig. 2B and C, white columns), as well as for its interaction with SRC1-Qr (Fig. 2B and C, black columns). Western blot analysis of extracts containing AR-NTD/Tau-5-DBD was done using a monoclonal M2 anti-Flag antibody. No difference in protein expression levels was observed (Fig 2B and C).

To validate the interaction of Tau-5 with SRC1-Qr, we did GST pull-down experiments. Bacterially expressed GST or GST-SRC1-Qr, immobilized on glutathione-Sepharose beads, were incubated with...
core Tau-1 and Tau-5 in AR-Mediated Transactivation

in vitro translated and [35S]methionine-labeled wtAR-NTD-DBD, a fragment containing only the Tau-5 region and the AR-DBD (AR-Tau-5-DBD) and a AR-NTD-DBD construct lacking the Tau-5 domain (AR-NTDΔTau-5-DBD; Fig. 2D). Besides the wtAR-NTD-DBD, only the fragment of the NTD encompassing the Tau-5 domain showed an interaction with SRC1-Qr, whereas the deletion mutant AR-NTDΔTau-5-DBD is not able to show an interaction with SRC1-Qr.

To correlate the interaction of SRC1-Qr with AR-Tau-5 with the coactivation ability of SRC1e, the expression constructs pSG5, pSG5wtAR, pSG5AR-ΔTau-5, pSG5AR-ΔTau-1, and the rTAT-GRE(E1b)-Luc reporter construct were transiently cotransfected into COS-7 cells (Fig. 2E, top). The wtAR is clearly coactivated by SRC1e, but when Tau-5 is deleted (AR-ΔTau-5), no coactivation is seen. However, SRC1e coactivation is possible when the first part of the AR-NTD is deleted, resulting in an AR fragment still containing the Tau-5 region (AR-ΔTau-1, aa 360-919). Moreover, cotransfection of wtAR and AR-ΔTau-1 with a SRC-1e construct containing mutated LxxLL motifs in its nuclear receptor interaction domain (SRC-1e M123) shows that the observed increase in activity is due to the Tau-5 interaction and not to the AF-2 in their LBD (Fig. 2E).

Western blot analysis of AR constructs shows no difference in expression level.

Comparison of Tau-1 of the hAR with τ1 core of the hGR. Simental et al. (23) and Pulvimo et al. (24) described the regions between aa 141 and 338 or aa 147 and 296 in human and rat AR-NTD, respectively, as essential for transcriptional activity. In the human GR, the major transactivation domain, called τ1, has been identified as a 185-amino-acid long region, containing a τ1 core region, which retains 60% to 70% of the activity of the intact domain (25, 26). This τ1 core domain contains three putative α-helical segments with the first two as the most important ones. In the AR-NTD, three segments form putative α-helices: helix 1 at position aa 177 to 185, helix 2 at position aa 187 to 199, and helix 3 at position aa 231 to 238 (Fig. 3A).

We have studied the effect of mutations in the three helices on the transactivation activity of the full-length AR in transient transfection experiments (Fig. 3B). A first construct contains the double mutation L181 and L192 to alanine (AR(M1)). In the second mutant AR, L196 and L197 are mutated into an alanine (AR(M2)). The third mutant is the combination of these four substitutions (AR(M4)). In the fourth construct, K234 and E235 are mutated to glycine, leading to the loss of the predicted α-helical structure (AR(M4)). Western blot analysis of extracts containing wtAR or AR mutants shows no difference in protein expression levels. The separate mutations in helices 1 and 2 lead to a >2-fold decrease in AR activity on the TAT-GRE elements, whereas AR(M5) shows even a slight increase in activity. Furthermore, the combination of the mutated helices 1 and 2 leads to a 5-fold reduced activation potential. This region is conserved among AR from different species (Fig. 3C). We will now call the two first helices the core Tau-1 activation domain of AR.

Mutation analysis of core Tau-1 of the hAR. To assess the relative importance of the core Tau-1 in AR activity, we did a mutation analysis of helices 1 and 2 in the full-size AR (Fig. 3D). Helix 1 has been predicted to form an acidic amphipathic α-helix (9, 27, 28). From Fig. 3B and D, it is obvious that the hydrophobicity and the helical structure correlate with activity because alanine and asparagine substitutions at positions L178, L183, L192, and L191 (Fig. 3D) have decreased AR activity. Alanine substitutions of K179 and S183 (AR(M7), AR(M8), and AR(M22)) lead to an increased AR activity. Although single mutations of the charged amino acids D177, D180, and E184 (AR(M5), AR(M8), and AR(M10)) do not have an effect on AR activity, triple mutation of these charged residues into alanines (AR(M22)) lead to a 2-fold decrease. The overall results obtained in HeLa cells are similar to what is shown for COS-7 cells in Fig. 3D (data not shown).

Western blot analysis revealed no difference in expression levels for wtAR or any of AR mutants. Furthermore, gel shift assays showed similar DNA binding for all constructs to the TAT-GRE element, and we could not detect changes in cellular localization and hormone binding (data not shown).

Core Tau-1 is an autonomous transcriptional activation domain. Because the core Tau-1 is involved in the transactivation by AR, we tested whether this region might contain an autonomous activation function by fusing it to the Gal4DBD and testing its
activity on a luciferase reporter gene controlled by Gal4 response elements (Fig. 4A and B). The core Tau-1M20, core Tau-1M21, and core Tau-1M22 mutations corrupt transactivation, whereas the core Tau-1M23 mutation enhances the transactivation potential of core Tau-1 by >3-fold. This correlates very well with the effect of these substitutions in the full-size AR (Fig. 3D). Fusion of more than one copies of core Tau-1 to the Gal4DBD leads to a gradual increase in intrinsic activity when tested in the mammalian one-hybrid assay (Fig. 4C).

Involvement of core Tau-1 in the interaction of the NTD with AR-LBD. It has been proposed earlier that an LKDI1-motif, here helix 1 of core Tau-1, also plays a role in the N/C interaction (9). To test the involvement of core Tau-1 in these interactions, a two-hybrid assay was done with mutant AR-NTDs fused to the VP-16 activation domain (Fig. 5A). Clearly, all AR constructs with negatively charged residues in helix 1 (AR-NTDM22) into alanines show a decreased interaction ( ~ 50%). Furthermore, AR-NTDM22 and AR-NTDM24 show an increase in N/C interaction.

Core Tau-1 controls the interaction of SRC1-Qr with the Tau-5 domain of AR. We subsequently tested whether the core Tau-1 activity is due to a possible interaction with SRC1-Qr in a mammalian two-hybrid assay. Wild-type AR-NTD and fragments were fused to the VP-16 transactivation domain and coexpressed together with the SRC1-Qr activation domain to the Ga/HD domain (Fig. 5B). As expected, the AR-NTD interacts well with SRC1-Qr, whereas the first part of the AR-NTD (AR-NTD aa 1-360) and core Tau-1 do not interact. However, deletion of core Tau-1 positively influences the interaction of Tau-5 with SRC1-Qr. Western Blot analysis of the constructs revealed equal expression of the different constructs.

To analyze this interplay in more detail, we tested mutations in the core Tau-1 region for their effect on binding of AR-NTD with SRC1-Qr (Fig. 5C). The luciferase expression in the two-hybrid assay clearly indicates a comparable interaction between the AR-NTDs with substituted hydrophobic residues (AR-NTD M1-3, AR-NTD M17, and AR-NTD M20-21) and SRC1-Qr compared with wtAR-NTD. There is only a small increase when E184 was substituted in AR-NTD (AR-NTD M17D). Strikingly, the constructs AR-NTD M5 and AR-NTD M8 show a 2-fold stronger interaction. These effects seem synergistic because substitution of all negatively charged residues in helix 1 (AR-NTD M22D) into alanines leads to a striking 13-fold induction compared with the wild-type fragment. The observed effects can not be explained by differences in protein levels.

Both core Tau-1 and Tau-5 are indispensable for intrinsic activity of the AR-NTD and for full AR activity. We already showed a strong autonomous function for Tau-5 (Fig. 2A) and core Tau-1 (Fig. 4B). From Fig. 6A, it is clear that both core Tau-1 and Tau-5 domains contribute to the full intrinsic activity of the AR-NTD. A fusion of the AR-NTD to the Gal4DBD domain shows a
strong constitutive transcription activation property, and deletion of Tau-5 (AR-NTDΔTau-5) does not abolish activation potency completely. Interestingly, an almost 2-fold decrease in luciferase activity is seen when helices 1 and 2 of core Tau-1 are mutated (AR-NTDM3). Combining both (AR-NTDM3ΔTau-5) leads to a construct lacking any intrinsic activation potency. Again, the observed effects are not due to differences in protein levels (Fig. 6A).

That core Tau-1 and Tau-5 cooperate for the AR-NTD intrinsic activity is also reflected in Fig. 6B. Fusion of both Tau-1 and Tau-5 to the DBD of AR leads to a luciferase activity higher than observed for the Tau-5-DBD construct. In addition, the more copies of the core Tau-1 domain fused to the Tau-5-DBD protein, the higher the activity observed. More importantly, also in the full-length AR, both an intact core Tau-1 and an intact Tau-5 are necessary (Fig. 6C). Indeed, mutation of core Tau-1 (ARΔM3) or deletion of Tau-5 (ARΔTau-5) each lead to a 5-fold decrease in AR activity. Combining both mutations (ARΔM3ΔTau-5) inactivates AR completely (Fig. 6C). Western blot analysis revealed no difference in expression levels for wtAR or any of AR mutants. Furthermore, the mutations did not result in changes in cellular localization or nuclear translocation (Fig. 6D).

Discussion

Prostate cancer mutations in the AR gene. Somatic mutations in the AR gene have been described in prostate cancer.1 Here, we report that the lysine-to-arginine mutation at position 179 described in a primary prostate cancer biopsy (19) results in a more potent AR. This is not due to a defect in acetylation because mimicking acetylation by the introduction of a glutamate also results in a more potent AR. Substitution by alanine has a similar potentiating effect (Fig. 1). Therefore, other posttranslational modifications, like methylation or ubiquitylation, or changed interactions with coactivators, or between the NTD and the LBD, could explain this observation. We have analyzed in more detail the activation domains within the NTD in an attempt to unravel its mechanisms of action.

Transcription activation function Tau-5. The large NTD of AR (529 amino acids long) contains a ligand-dependent activation function, called Tau-1, and a constitutively active activation domain, called Tau-5 (18). From a deletion analysis, we conclude...
that the integrity of the complete Tau-5 extending from position 360 to 529 is required for its optimal autonomous activation function (Fig. 2A). The relative importance of Tau-5 for the AR-NTD activity depends on the response element or promoter used in the assay, pointing to a changing relative importance of Tau-1 and other activation functions within the AR-NTD. Similarly, the importance of the N:C interaction and of the SUMOylation of AR at position aa 385 varies according to the enhancer tested (15, 29).

It was described earlier that AR recruits p160 coactivators through an interaction between the AR-NTD and a glutamine-rich domain within the p160s (9–12). Here, we show that the integrity of Tau-5 is a prerequisite for p160 recruitment (Fig. 2B). Indeed, a deletion of Tau-5 in the AR or AR-NTD prevented coactivation by SRC1, whereas coactivation is maintained for AR constructs still containing the Tau-5 domain (Fig. 2C-E).

**Defining core Tau-1 in the hAR.** The molecular mechanism of action of Tau-1, the ligand-dependent activation function of the AR-NTD, and that of other nuclear receptors remains obscure. The NH2-terminal transactivation domains of steroid receptors are the least conserved domains; hence, each is believed to act through alternative mechanisms. For several receptors, putative helices have been suggested as important for the NTD AF-1 function.

---

**Figure 3. Core Tau-1 domain of the hAR.**

**A,** comparison of the domain structure of the H1 core of the hGR with core Tau-1 of the hAR. The residues with a high probability for α-helical formation (helices 1-3 for the GR and AR) are in boldface. Numbers indicate the amino acid positions of the helices. The amino acids that were mutated into alanines are underlined. **B,** study of core Tau-1 in the hAR. COS-7 cells were transfected with constructs expressing wtAR or mutated ARs (10 ng/well) together with the rTAT-GRE luciferase (100 ng/well) and the CMV-hGal (5 ng/well) reporter constructs. Cells were stimulated with or without 10⁻¹⁰ M R1881 for 24 hours before analysis (open and black columns, respectively). Activities are depicted relative to the activity of the wtAR construct in the presence of hormone, which was set on 100. Columns, mean; bars, SE. Western blot analysis of the cell extracts containing wtAR and AR mutants using anti-Flag antibody were done as described in Materials and Methods. **C,** sequence alignment of core Tau-1 of different species. Residues in boldface represent the high sequence identity of helix 1. **D,** mutation analysis of core Tau-1. The transfection and the Western blot were done as described in Fig. 3B. Helix1 is represented in a helical wheel.
Because the Lys179 mutation into arginine resulted in a more active AR and because it is situated in a conserved domain in the AR-NTD (Fig. 3C), we did a mutation analysis of the putative α-helices surrounding this lysine. This led to the definition of a two-helical core Tau-1, which is mandatory for proper AR functioning (Fig. 3A and B).

**Mutation analysis of Tau-1.** Three α-helices are predicted near Lys179 (Fig. 3A). In the rat AR, helix 1 was called AF-1a by Chamberlain et al. (27). Besides AF-1a, Chamberlain et al. identified a second transactivation region called AF-1b, which resembles an acidic activation domain. Although this sequence is highly conserved among AR of different species, a deletion of the corresponding fragment (292-351) in the hAR did not alter its transactivation capacity in transient transfections (data not shown). Mutation analysis of helix 1, however, revealed the importance of this region for AR functioning (Fig. 3D).

The mutation analysis of core Tau-1 reveals that both the hydrophobic side chains and the negatively charged side of the amphipathic helix 1 are important. Surprisingly, the residues K179 and S183 (M23) seem to have a repressive effect because their mutation resulted in a much more potent AR (Fig. 3D). We also showed the importance of L190/L191 in helix 2, as well as its α-helical structure for the Tau-1 function (Fig. 3). We observed a small increased AR activity when helix 3 is mutated (Fig. 3A). The latter is in agreement with studies of the group of Greenberg, who observed an enhanced prostate cancer development in transgenic mice expressing an AR-K231G mutant (32).

Taken together, we define the core Tau-1 within the NTD of AR as a two-α-helix-containing fragment with a central role in the proper functioning of the hAR. Core Tau-1 is well conserved among mammals, but in *Xenopus laevis* and *Rana catesbeiana* AR, only the first α-helix of core Tau-1 is conserved, and in fish AR, core Tau-1 seems completely absent (Fig. 3C).

Although Tau-1 was initially described as a ligand-dependent activation function within the AR-NTD, a chimerical protein consisting of core Tau-1 fused to the Gal4DBD activates transcription up to 40% of that observed for a AR-NTD fused to Gal4DBD (Fig. 4). This shows that, next to the autonomous core Tau-5, also core Tau-1 is an important autonomous activation function. The structure-function relationships within this autonomous function is identical to that in the ligand-dependent function, because mutation analysis of core Tau-1 resorted in nearly identical effects in the full-length AR and in the Tau-1 Gal4DBD fusion (Fig. 4B). The mutation of K179 and S183 results in an almost 4-fold more active AR (Fig. 3A) and a 3- to 4-fold more active autonomous Tau-1 (Fig. 4B). Furthermore, the transactivating properties of the fusion of
Figure 5. A, two-hybrid assay for the interaction between AR-NTD and AR-LBD. pSG5AR-DBD-LBD (aa 538-919; 50 ng/well) was coexpressed in COS-7 cells with either the empty pSNATCH-II expression vector encoding the activation domain of VP16 or the same expression construct containing the wild-type NTD or mutant NTDs fused to it (50 ng/well). Assays were done using the 2TAT-GRE(E1b)-Luc reporter (100 ng/well) and the CMV-hGal reporter (5 ng/well) in the presence or absence of 10^-8 M R1881. Activities are depicted relative to the activity of the wtAR-NTD construct, which was set to 100. Columns, mean; bars, SE. B-C, effect of core Tau-1 on the interaction of SRC1-Qr with Tau-5. Deletion mutants in the hAR-NTD and their interaction with SRC1-Qr. pABGal4-DBDSRC1-Qr (989-1240; 50 ng/well) was coexpressed in COS-7 cells with 50 ng of either the empty pSNATCH-II expression vector (open columns) or the same expression vector containing the wild-type AR-NTD or the indicated deletion mutants (50 ng/well; black columns). Assays were done using the (Gal4) 5-TATA-Luciferase reporter (100 ng/well) and the CMV-hGal reporter (5 ng/well). Columns, mean; bars, SE. Western blot analysis of the cell extracts using anti-Flag antibody were done as described in Materials and Methods [lane 1, AR-NTD; lane 2, AR-NTDΔTau-5; lane 3, AR-NTD Δcore Tau-1; lane 4, core Tau-1 (aa 173-203)]. Since core Tau-1 fused to the Gal4DBD is a small fragment, a longer exposure was necessary. C, the same analysis for mutated AR-NTDs as done in (B).
multiple copies of Tau-1 to the Gal4DBD increased with the number of Tau-1 copies, indicating its independent function (Fig. 4C).

Functional relations between different domains of AR and SRC1-Qr. A strong N:C interaction has been correlated with AR function, mainly through the FQNLF-motif at the NH2-terminal end of AR, and the hydrophobic cleft in the AR-LBD (9, 13–16, 33). Surprisingly, some mutations of helix 1 and 2 of core Tau-1 led to a 2-fold decreased N:C interaction, even when the FQNLF motif is present in the construct (Fig. 5A; ref. 9). Mutations enhancing the hydrophobic nature of the surroundings of the LKDIL motif enhanced the N:C interactions (Fig. 5A). This means that not only the LKDIL motif but also residues in core Tau-1 are involved in this interaction. However, because the deletion of the FQNLF-motif abolished the N:C interaction (15), core Tau-1 can only be a secondary interaction site for the LBD.

Deletion of Tau-5 abolished the p160 coactivation of the full-length AR (Fig. 2E) or the AR-NTD (Fig. 5B) almost completely. Although the isolated Tau-1 does not interact with the glutamine-rich region of SRC1 (Fig. 5B), the deletion of core Tau-1 in the AR-NTD enhanced the SRC1-Qr recruitment to the NTD >3-fold. Mutation analysis of the core Tau-1 for changes in the interactions between the NTD and SRC1-Qr led to the observation that some mutations indeed increased this interaction (Fig. 5C). The mutation of the three negatively charged residues in helix 1 (ARM22) increased the interaction 13-fold. This mutation in the full-length receptor did not increase the androgen response in COS-7, CHO-K1, and HEK293 cells, but a 2-fold increase in AR activity of ARM22 was seen in HeLa and U-2OS cells (data not shown), indicating that cell-specific factors may be involved. Mutation of the positively charged amino acids (ARM24) led...
to both an increased SRC interaction and an increased AR activity.

In conclusion, although Tau-1 is not a primary interaction site for p160 coactivators, it does affect the p160/Tau-5 interactions. Because there is no direct interaction of core Tau-1 with SRC1, and no interdomain interactions between Tau-1 and Tau-5 (data not shown), this effect must be indirect (e.g., via induction of a conformational change), or the recruitment of a secondary interaction partner. If the role of core Tau-1 would only be the induction of an activating change of conformation in Tau-5, adding multiple copies of core Tau-1 should not have an additional effect on Tau-5 fused to Gal4DBD. However, the transactivating properties of these fusions increased with the number of core Tau-1 copies. These results might, therefore, be explained by the involvement of another coactivating partner for Tau-1. Although several candidate coactivators for AR AF-1 have been reported (34), they should now be tested against the mutations in core Tau-1 described here.

Tau-1 and Tau-5 explain the transactivating properties of AR. The AR-NTD is a very potent activation domain. When core Tau-1 or Tau-5 are mutated, its potency is diminished, and when both mutations are combined, the NTD is inactivated (Fig. 6A). Interestingly, the fusion of one copy of each Tau-1 and Tau-5 led to a transcription factor more potent as the Tau-1 alone, indicating that the two Taus are the major players in the transactivation by this domain (Fig. 6B).

When Tau-1 and Tau-5 mutations are introduced in the full-length AR, it is almost completely inactivated (Fig. 6C), indicating their important role in transactivation and also illustrating the weakness of the AR AF-2.

In conclusion, we propose the following steps in AR induction of transcription (Fig. 6D). AR-LBD recognizes ligand and induces the nuclear translocation and DNA-binding of AR to either classic or selective ARES (35). The absence of an activation function in the AR-LBD is probably explained by the strong intramolecular N/C interaction, mediated via the FQNLF motif and enhanced by the LKDLL motif. This interaction prevents the recruitment of LxxLL-containing coactivators to the AR-LBD but stabilizes the interaction of the agonist with AR (36, 37). It is a new finding that for the full activity of AR, a concerted interplay between Tau-1 and Tau-5 is necessary and sufficient to explain the transactivation, Tau-5 is the recruitment surface for the p160s. We have assigned an autonomous transactivation function to a core Tau-1, which is also indirectly involved in the recruitment of p160s to Tau-5. This model explains the enhanced transactivating properties of mutant ARs described in some prostate cancer biopsies and indicates the existence of an additional non-p160 coactivator, which acts through Tau-1.

Acknowledgments

Received 7/8/2005; revised 9/21/2005; accepted 10/19/2005.

Grant support: Geconcerteerde Onderzoeksactie van de Vlaamse Gemeenschap, Fonds voor Wetenschappelijk Onderzoek, Vlaanderen, Association for International Cancer Research, and Congresonally Directed Medical Research Program: Prostate Cancer Research Program award DAMD17-02-1-0092. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

We thank R. Bollen, H. Debryns, and K. Bosmans for their excellent technical assistance and our colleagues of the Molecular Endocriology Laboratory for helpful discussions.

References


Downloaded from cancerres.aacrjournals.org on April 19, 2017. © 2006 American Association for Cancer Research.


Interplay between Two Hormone-Independent Activation Domains in the Androgen Receptor

Leen Callewaert, Nora Van Tilborgh and Frank Claessens


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/66/1/543

Cited articles
This article cites 36 articles, 22 of which you can access for free at:
http://cancerres.aacrjournals.org/content/66/1/543.full.html#ref-list-1

Citing articles
This article has been cited by 12 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/66/1/543.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.