Advances in Brief

Ligand Responsiveness in Human Prostate Cancer: Structural Analysis of Mutant Androgen Receptors from LNCaP and CWR22 Tumors

Shawn McDonald, Lars Brive, David B. Agus, Howard I. Scher, and Kathryn R. Ely

Cancer Center, The Burnham Institute, La Jolla, California 92037 [S.M., L.B., K.R.E.], and Memorial Sloan-Kettering Cancer Center, New York, New York 10021 [D.B.A., H.I.S.]

Abstract

Androgen receptors (ARs) belong to the family of hormone receptors that are ligand-dependent transcription factors. Endocrine therapy provides effective treatment for prostate cancer until mutations arise that alter the ligand responsiveness of AR. In this study, structural models were developed for the functional domains of human AR by homology modeling from crystal structures of closely related nuclear receptors. These models were used to locate the sites of two frequently occurring mutations in prostate cancer. The substitutions that develop in LNCaP (threonine→alanine at residue 877) and CWR22 (histidine→tyrosine at residue 874) tumor cell lines are both located on helix 11 that forms part of the ligand-binding pocket. However, the results suggest that these mutations influence ligand responsiveness by completely different mechanisms. Residue 877 contacts the ligand directly, and substitution at this site alters the stereochemistry of the binding pocket. Thus, the LNCaP mutation apparently broadens the specificity of ligand recognition. In contrast, residue 874 is located down the helical axis, projects away from the ligand pocket, and does not contact ligand. The side chain of residue 874 lies in a cavity between helices 11 and 12. Substitution of tyrosine for histidine 874 in CWR22 tumors may affect a conformational change of helix 12 and, thus, influence binding of coactivator proteins and their regulatory effect on transcriptional activation.

Introduction

Endocrine therapy has been an accepted treatment for metastatic prostate cancer for more than 50 yr. Because prostate cancer is androgen dependent in the initial stages of the disease, treatment is aimed at blocking production of testosterone and other androgens that serve as ligands for the AR. Androgen withdrawal produces significant palliation of symptoms in patients with advanced prostate cancer. However, on close inspection, the proportion of tumors that actually regress is small, and the majority of patients show biochemical, radiographic, and clinical progression to an androgen-independent phenotype that is ultimately lethal for the patient. It has been thought that androgen-independent proliferation (growth in the setting of castrate levels of testosterone) represents hormone refractory disease, and the hypothesis was that after androgen withdrawal AR-mediated signaling no longer contributes to proliferation. Recent clinical and laboratory investigations challenge this view and point to AR-mediated signaling, even with only castrate levels of testosterone. Evidence for the importance of AR-mediated signaling in advanced-stage disease is accumulating from clinical studies with antiandrogens after androgen withdrawal (2) and from analyses of the frequency of AR gene amplification and mutation rate in prostate tumors (3–8).

The AR belongs to the superfamily of steroid/nuclear receptors that are ligand-dependent transcription factors. Other members of the family include receptors for thyroid, glucocorticoid, retinoic acid, estrogen, and progesterone hormones. The receptors are multifunctional proteins that contain transactivation domains, as well as DBDs and LBDs. The domains are independently folded structural modules (see Fig. 1) linked by extended polypeptides. Three-dimensional structures for the DBDs or LBDs of a number of these receptors, determined by X-ray crystallography or nuclear magnetic resonance, reveal a common folding pattern across the family, as predicted by conserved amino acid sequences. Interestingly, the ligands for this family of receptors, the steroid hormones, are also closely related in structure. Yet, the individual receptors discriminate between ligands, with stereochemically specific recognition, and accurately activate specific genes in response to binding selected ligands.

Two mutant ARs have been observed repeatedly in studies of human prostate cancer. The first is a mutation at residue 877 that substitutes Ala for Thr and occurs in LNCaP prostate tumor cell lines (9, 10). This tumor line was derived from a human metastatic lesion of prostate carcinoma, and the tumor cells respond to androgen and grow in culture. The other mutation, His874Tyr, was reported as a somatic mutation in tumors from patients with advanced androgen-independent prostate cancer tissues after androgen ablation (8). A human prostate cancer xenograft, CWR22 (11, 12), bears the His874Tyr mutation in the LBD. These point mutations at residues 877 and 874 have also been reported in patients with androgen-independent metastatic prostate cancer (8) from advanced-stage prostate tumors, from tumors after androgen ablation (13, 8), or in androgen-independent tumors that develop from CWR22 tumors after androgen withdrawal (14). Mutant ARs with these interchanges exhibit altered ligand specificity and are responsive to adrenal androgens, estrogens, or progesterones. It has been proposed that these altered ARs may respond to other ligands to stimulate growth of prostate tumor cells after androgen withdrawal.

In this study, three-dimensional homology models of the human AR were developed to map the sites of these mutations that are observed in relapsed androgen-independent disease and in human prostate cancer xenografts. Both mutations occur in the LBD, yet one is expected to alter the shape of the ligand-binding pocket and influence ligand recognition, whereas the other is proposed to modulate a conformational adjustment away from the binding pocket and affect binding of coactivators. The models offer the capability to structurally characterize distinct mechanisms of progression in advanced prostate cancer.

Materials and Methods

Homology Modeling. Sequence databases (PDB and PIR) were searched for sequences related to the human AR sequence (NCBI accession code P10275; PID g113830; Ref. 15) using PSI-BLAST (16) and T-98 HMM (17).
The six most closely related sequences were aligned with ClustalW (18) using default parameters. Homologous sequences for domains with known crystal structures were selected as templates to construct the models: rat glucocorticoid receptor for the DBD (AR residues 559–624; numbering as in Ref. 15) and human PR for the LBD (AR residues 668–919). Coordinates for the atomic models were retrieved from the Protein Data Bank: DBD (accession code 1GLU; Ref. 19) and LBD (accession code 1A28; Ref. 20). Pairwise sequence alignments for the template and each appropriate human AR domain were visualized using the program ALSCRIPT (21), displaying the sequences along with secondary structural features of the templates. These alignments were used as input for automated homology modeling with the program MODELLER (22) to create the two homology models. Through MODELLER, the residues of the models were changed to sequences of AR at homologous sites. Polar hydrogens were added to the model in QUANTA 97.0 (Molecular Simulations, Inc., San Diego, CA).

From this point, different protocols were used for the two domains. For the DBD, the model was relaxed by energy minimization using CHARMM within QUANTA (23). Close contacts or buried hydrophilic side chains were adjusted manually, and then the process was repeated: first 200 steps of steepest descents minimization, followed by 600 steps of conjugate gradient minimization (final rms force for DBD = 0.23 kcal/mol-Å). For the LBD, a different strategy was used to avoid conformational “collapse” of the ligand pocket during energy minimization. A model of testosterone was manually docked as a rigid body to the binding site guided by an overlay of the steroid rings onto progesterone in the pocket of PR from the crystal structure. Side chains within 8 Å of any ligand atom were inspected. Two residues (Arg752 and Met749) were adjusted to avoid steric contacts, guided by homologous residues in PR and established rotamer libraries (24). Twelve residues were noted as deviating from ideal geometry, and these residues were adjusted manually using rotamer libraries and visual comparison with PR.

The geometry and stereochemistry of the final models were checked with PROCHECK (25) and with the Protein Health module of QUANTA 97.0. These analyses indicated that 99% (LBD) and 91% (DBD) of nonglycinyl residues fall within allowed regions in the Ramachandran diagram. Stereochemical parameters fell within allowable limits with maximal values for bond length and bond angle rms deviations of 0.03Å and 2.9° for the DBD model and 0.017Å and 2.9° for the LBD model. Images of the models presented in the figures were prepared with QUANTA, SPOCK (26), and Raster3D (27).

Results and Discussion

Structural Models. The homology models of AR DBD and LBD are shown in Fig. 1. The homology models reported here were based on known crystal structures of structurally related steroid-hormone
receptors. This was necessary because no atomic structures are available for AR domains from crystallographic or nuclear magnetic resonance studies. Coordinates from the atomic models of the DBD of glucocorticoid receptor (PDB code 1GLU; Ref. 19) and the LBD of PR (PDB accession code 1A28; Ref. 20) were the template coordinate files. In another study, a homology model was developed for the AR DBD, also from the glucocorticoid receptor (28). The sequences of the templates and the AR domains are strongly homologous (52% and 82% identical between receptors for LBD and DBD, respectively). The sequence alignment of PR and AR LBD is shown in Fig. 2. The sequences aligned directly without the introduction of gaps. There is also a high degree of structural homology in related receptors. The position of ligand within the binding pocket of PR, determined by crystallographic analysis, was used to dock testosterone into the binding pocket of the AR homology model. It is apparent from this comparison, shown in Fig. 3, that the ligand pockets are closely related, yet the residues lining the pocket and stereochemistry of the crevice are unique to each receptor.

**Mutations in Prostate Cancer Cell Lines.** The substitutions that develop in LNCaP (Thr→Ala at residue 877) and CWR22 (His→Tyr at residue 874) tumors were considered independently and in the context of the tabulation from the database. These mutations are both located on one helix in the LBD that forms part of the ligand-binding pocket. Residues on one face of this helix interact with ligand (helix 11; see numbering of helices in PR in Ref. 20). AR residue 877 corresponds to Cys891 in PR that contacts the ligand. In contrast, AR residue 874 is three residues away around the helical axis and projects away from the ligand pocket. The residue corresponding to AR His874 is also His in PR and does not contact the ligand. The residue adjacent to His 874 is a conserved Leu (Leu887 in PR). In PR, this leucine is found in the pocket and makes van der Waal’s contacts with the ligand. The sites of these mutations are shown in Fig. 4, and the structural framework for their effect in ligand recognition or transactivation is discussed in detail in the following sections.

**Mutations and Receptor Specificity.** Threonine at position 877 limits specificity of AR to androgens, but substitution of other small amino acids at this site reduces the binding specificity for steroids (29). Thr877 is located in the binding pocket, and substitution by alanine (see Fig. 4) apparently alters the available space in the hydrophobic pocket so that the specificity of such mutant ARs is...
broadened to include progestagens, estrogens, and antiandrogens (10). Interestingly, the residues at this site in related receptors differ, with Cys891 in PR and Leu525 in ER. The molecular basis for recognition of specific steroids is complex. Direct contacts with the 3-keto group on the steroid in the progesterone/PR complex (20) involves two conserved residues: glutamine 725 from helix 3 (residue 711 in AR) and arginine 766 (residue 752 in AR) from helix 5. These interactions are stereochemically permitted in our AR model and represent a generalized mode for nuclear receptors to bind steroids with 3-keto substitution at C3. Specific recognition of ligands also requires interaction with substituents at C17 on the steroid nucleus. At the opposite extreme of the ligand pocket, residues extending from helix 11 near T877 are in close proximity to the C17 ligand substituents in complex with PR and ER (20, 30, 31).

The Thr877Ala mutation was found in 5 of 16 patients who had received androgen blockade therapy consisting of androgen withdrawal and the AR antagonist flutamide (8). Similarly, the original LNCAp cell line was derived from a patient who was undergoing treatment with androgen ablation after orchiectomy and estrogen treatment (9, 10). ARs with the mutation seen in LNCaP cells, or that lack Thr at residue 877, exhibit increased responses to estradiol and progesterone as compared with wild-type AR (8, 10, 29). Yet, recognition of androgens is not lost when alanine is substituted at residue 877. LNCaP cell lines bearing the Thr877Ala mutation are still responsive to androgen, and growth inhibition occurs after androgen withdrawal. In addition, it is clinically relevant that mutant ARs with substitutions at residue 877 are stimulated by hydroxyflutamide, the active metabolite of flutamide, to a greater extent than wild-type AR (32, 33). It has been proposed that mutated ARs may be a mechanism whereby prostate cancers become refractory to treatment with androgen withdrawal. Mutant ARs with altered ligand-binding pockets could contribute to tumor progression after androgen withdrawal if the mutant receptors stimulate cell growth in response to other ligands.

Mutations and Ligand Responsiveness. Mutant receptors with the His874Tyr substitution are transcriptionally active, and the response to testosterone and hydroxytestosterone is similar to that of wild-type ARs (33). But, in contrast to wild-type AR, the His874Tyr mutant is stimulated by adrenal androgen (dehydroepiandrosterone),

---

Fig. 4. Sites of LBD mutations in AR from LNCaP cell lines and CWR22 tumor cells. This figure presents a “close-up” comparative view of the substitutions, with the ligand testosterone docked for orientation. As in Fig. 3, some residues have been removed to view sites at the interior of the domain. The CPK models are gray, and the highlighted side chains are light blue, nitrogen atoms are blue, oxygen atoms are red, and testosterone is yellow. The substituents at C3 and C17 on the ligands are located in bottom and top positions, respectively, in the images. a. Thr877 is highlighted and compared with an alanine substituted at this site, as shown in b. Substitution of a residue with a smaller side chain that is not β-forked would enlarge the pocket in this region. In PR a cysteine in the equivalent position is in van der Waal’s contact with the ligand. c. His874 is highlighted and compared with a tyrosine at this site, as shown in d. This residue is adjacent to Leu 873, which corresponds to a Leu in the PR that makes van der Waal’s contact with the ligand. However, as can be seen in the images, His874 does not contact the ligand and is not located in the ligand-binding pocket. There is ample space to accommodate the larger tyrosine side chain found in the mutant ARs so that no conformational changes are expected with this substitution. Instead, it is possible that this mutation interacts with coactivators and transcriptional activity.
estradiol, progesterone, and flutamide, as measured by transcriptional response (32, 33). The Thr877 LNCaP mutation discussed in the previous sections is, based on the proposed model, expected to alter ligand specificity by changing the shape of the binding pocket. In contrast, residue 874 is not in direct contact with the ligand and the side chain points away from the binding pocket (see Fig. 4). Therefore, the His874Tyr mutation in CWR22 tumors most likely influences ligandresponsiveness by a completely different mechanism.

Nuclear receptors are transcriptionally active in response to binding hormone. Coactivator proteins enhance this ligand-dependent activity. Binding of coactivator is promoted by receptor agonists and blocked by antagonists, and there is a conformational difference in the receptors in these two states (30, 34, 35).

The crystal structure of human ERs has been determined bound to the agonist diethylstilbestrol and a peptide from the coactivator protein p160 GRIP1 (NRII), and this structure was compared with ERα bound to the antagonist 4-hydroxytamoxifen (30). In the agonist/coactivator complex, the recognition motif LxxLL (NR box) in NRII interacts with four residues that are highly conserved in the nuclear receptor family, as well as other hydrophobic and nonpolar contacts. When the 4-hydroxytamoxifen antagonist is bound to ERs, helix 12 assumes a different conformation that likely inhibits binding of coactivator. In this structure, as well as in a complex of ER with the antagonist raloxifene (31), helix 12 assumes a different conformation and binds to the crevice that accommodates the coactivator NR motif in the agonist-bound structure. The sequence LxxML in ER mimics the LxxLL motif in NRII. In ARs, the corresponding sequence is MxxII (residues 895–899), so a similar complementary interaction can be expected for binding of helix 12 in AR to the coactivator pocket.

The His874Tyr mutation observed in prostate cancer may affect the conformational adjustment of helix 12 and, thus, influence coactivator binding (reviewed in Ref. 36). The side chain of residue 874 lies in a buried cavity between helices 11 and 12 that is formed when helix 12 is in the agonist-bound conformation. There is ample space in the buried cavity to accommodate the tyrosine aromatic ring (see Fig. 4) and, thus, the substitution is not expected to produce a steric change at this site. This cavity also contains ordered water molecules in the PR structure. Change from a polar histidine to the more hydrophobic tyrosine side chain could affect the strength of binding of helix 12 to this crevice, resulting in a modification of the regulatory effect of coactivator on transcriptional activation. Future studies using mutagenesis are needed to evaluate this effect directly.

Concluding Remarks. In this study, we have produced molecular models of two functional domains of the human AR. The models were produced by homology modeling using crystal structures of closely related nuclear steroid-hormone receptors as templates. The models provide a framework to consider the function of ARs and to locate the sites of mutations that frequently occur in prostate cancer. Two mutations that are observed repeatedly affect ligand responsiveness: a Thr→Ala substitution at residue 877 in LNCaP cell lines and a His→Tyr substitution at residue 874 occurring in CWR22 human prostate tumor cells. It is likely that these two mutations influence ligand responsiveness by different mechanisms. These two mutations are expected to affect AR interactions with ligand (residue 877) or coactivator protein (residue 874). Understanding these mutations is vital to the strategic design of hormone-based treatments for the entire course of prostate cancer. With the homology models we have developed, the tools are now available to define the molecular basis for response in mutant ARs and to use the molecular data to develop future therapeutics targeted to mutant receptors that appear in late-stage prostate cancer.


Ligand Responsiveness in Human Prostate Cancer: Structural Analysis of Mutant Androgen Receptors from LNCaP and CWR22 Tumors

Shawn McDonald, Lars Brive, David B. Agus, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/60/9/2317

Cited articles
This article cites 33 articles, 14 of which you can access for free at:
http://cancerres.aacrjournals.org/content/60/9/2317.full#ref-list-1

Citing articles
This article has been cited by 11 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/60/9/2317.full#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.