18 Drugging the Undruggable: Targeting the N-Terminal Domain of Nuclear Hormone Receptors

Marianne D. Sadar

Abstract

This chapter focuses on the development of drugs targeting the N-terminal domain of nuclear hormone receptors, using progress with the androgen receptor as an example. Historically, development of therapies targeting nuclear hormone receptors has focused on the folded C-terminal ligand-binding domain. Therapies were traditionally not developed to target the intrinsically disordered N-terminal domain as it was considered "undruggable". Recent developments have now shown it is possible to direct therapies to the N-terminal domain. This chapter will provide an introduction of the structure and function of the domains of nuclear hormone receptors, followed by a discussion of the rationale supporting the development of N-terminal domain inhibitors. Chemistry and mechanisms of action of small molecule inhibitors will be described with emphasis on N-terminal domain inhibitors developed to the androgen receptor including those in clinical trials.

Keywords

Androgen receptor · Intrinsically disordered protein · Drugs · Ralaniten · EPI-002 · Sintokamide

18.1 Introduction

Nuclear hormone receptors share a common modular structural organization that includes a variable N-terminal domain (NTD or A/B domain), a DNA-binding domain (DBD or C domain), a non-conserved hinge region (D domain), and a C-terminal ligand-binding domain (LBD or E domain) [33]. Here we focus on members of nuclear receptor subgroup 3, that include the androgen receptor (AR), two closely related estrogen receptors (ERα and ERβ), glucocorticoid receptor (GR), mineralocorticoid receptor (MR), and progesterone receptor (PR). These are soluble proteins that mediate the effects of lipophilic steroids to regulate the expression of thousands of genes to control the growth and function of cells and tissues [36, 39, 70, 119]. Steroidal hormones diffuse across the cell membrane to bind to the LBDs of hormone receptors, which sets off a series of events that are necessary for transactivation or repression of target genes. First there is a conformational change of the receptor that involves the shedding of interacting chaperones

M. D. Sadar (✉)
Canada's Michael Smith Genome Sciences Centre, British Columbia Cancer, Vancouver, BC, Canada

Department of Pathology and Laboratory Science, University of British Columbia, Vancouver, BC, Canada
e-mail: msadar@bcgsc.ca

M. J. Campbell, C. L. Bevan (eds.), *Nuclear Receptors in Human Health and Disease*, Advances in Experimental Medicine and Biology 1390, https://doi.org/10.1007/978-3-031-11836-4_18

followed by translocation of the receptor to the nucleus. The DBD directs binding of the receptor to specific genomic regions on the DNA and dimerization, followed by recruitment of coregulatory proteins, chromatin remodelers, and the general transcriptional machinery necessary for regulating the transcription of target genes [12, 33, 36, 63, 70, 119]. There are two regions within the NTD and LBD called activation functions 1 and 2 (AF-1 and AF-2) respectively that provide the surfaces for interaction with coregulators and the transcriptional machinery [22, 34, 56, 62, 85]. To date, all clinically approved therapies directed against these hormone receptors target AF-2 in their LBDs. However, the recent breakthrough of the discovery of small molecule inhibitors that directly interact with AF-1 of AR has yielded the first ever small molecules that directly interact with the previously-considered "undruggable" NTD of a hormone receptor. The success of drug development against the intrinsically disordered NTD of AR is a precedent in the field of hormone receptors, but it is also worth noting that these molecules were the first drugs that directly bind to any intrinsically disordered target to reach human clinical trials (NCT02606123). Since success in drugging the "undruggable" NTDs of hormone receptors is currently restricted to AR, this review focuses on AR to provide insight into drug development that may have application for other nuclear hormone receptors.

18.2 Modular Structure of Nuclear Hormone Receptors

Nuclear hormone receptors are modular proteins. The steroid hormone receptors vary in size from less than 600 amino acid residues to over 900 residues. Their modular structure includes an intrinsically disordered N-terminal domain (NTD), DNA-binding domain (DBD), hinge region, and C-terminus ligand-binding domain (LBD). There can be substantial amino acid sequence similarity depending on the domain and hormone receptors being compared. For example, the AR-LBD shares 54% sequence similarity with PR-LBD and hence some antiandrogens can inhibit the transcriptional activity of PR [10, 84]; the AR DBD is 76% identical to that of the GR-DBD and not surprisingly they share some common regulatory DNA sequences within the same chromatin loci [20, 102]. This is an important consideration in drug development since the specificity of these hormone receptors involves multiple mechanisms including receptor-specific residues within their ligand-binding pockets but also importantly tissue-specific expression of a hormone receptor which, if not appreciated, could lead to unexpected toxicity in other tissues (for a review see [19]). For example, benign prostate tissue expresses AR but does not express GR, yet in advanced prostate cancer both GR and AR are expressed [52].

18.2.1 Intrinsically Disordered N-terminal Domain (NTD)

The NTDs of these hormone receptors have little sequence conservation (<15%) and vary enormously in size from only 182 amino acid residues for ERα to over 600 residues for MR. The NTD contains activation function 1 (AF-1), which interacts with an abundance of coregulatory proteins [42, 57, 64, 68]. AF-1 of the majority of hormone receptors contains most or all of the transcriptional activity, with the exception of ERα which has most of its transcriptional activity within AF-2 in its LBD [8, 22, 48, 56, 62]. AF-1 and AF-2 can act independently, as demonstrated with deletion and mutational experiments, but generally maximum activity is obtained when AF-1 and AF-2 cooperate in concert ([64, 81]). AF-1 is generally considered ligand-independent. However, AR AF-1 has two transactivation units -1 and 5 (tau-1 and tau-5, respectively) (Fig. 18.1). Tau-1 is considered to be dependent on ligand binding to the receptor and encompasses amino acid residues 101-370, the majority of which are acidic. Amino acid residues 360-485 comprise tau-5, which is considered to be ligand-independent. Deletions of small regions of approximately 100 residues of tau-1 do not eliminate AR transcriptional activity, suggesting that the activity of tau-1 is not attributable to a single

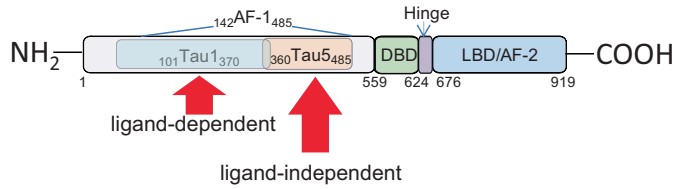

Fig. 18.1 Modular structure of the androgen receptor. AF-1 is within the N-terminal domain (NTD) and contains the ligand-dependent tau1 and ligand-independent tau5. The DNA-binding domain (DBD) contains 65 amino acid residues. The hinge region connects the DBD to the LBD and contains a nuclear localization signal. AF-2 is within the ligand-binding domain (LBD) and contains 249 amino acid residues

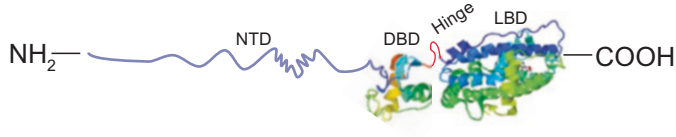

Fig. 18.2 Illustration of the AR displaying the intrinsically disordered NTD and hinge region compared to the folded DBD and LBD

small structural element [55]. This point is of relevance in drug development because it could mean that any small molecule inhibitor, or antibody, directed to tau-1 would need to impact the conformation broadly and not merely a small discrete region. Theoretically this would imply that the recently developed bispecific antibody 3E10-AR441, that binds within tau-1 at residues 299-315 [37], would not be efficacious in blocking all AR transcriptional activity. Additionally, loss of AR-LBD shifts the transcriptional activity of AR from tau-1 to tau-5 [55], which has implications for finding a small molecule inhibitor that blocks both full-length AR in response to ligand (activity mediated through tau-1) and truncated splice variants of AR (AR-Vs) that lack LBD (activity mediated through tau-5).

NTDs of hormone receptors are not amenable to structural analysis by X-ray crystallography due to their intrinsic disorder, thereby impeding drug development (Fig. 18.2). Amino acid residues dictate the disordered state and thereby are "intrinsic" to the coding sequence. Generally, intrinsically disordered proteins or regions are enriched in amino acid residues that have a high net charge, low hydrophobicity, and abundance of proline residues [30, 117, 118]. Cysteine residues can form disulfide bridges that stabilize the protein structure in an oxidizing environment, but under a reducing environment, the disulfide bridges are broken, resulting in the protein becoming less ordered. Figure 18.3 shows a Ronn plot that predicts regions of disorder within the AR-NTD based upon its amino acid sequence. Post-translational modification such as phosphorylation also impacts intra- and intermolecular interactions [7], which in turn impact the conformation of the structure and binding partner preference, plus the protein half-life [23, 40, 124]. Aromatic residues may reveal a molecular recognition region (MoRF) within a region of intrinsic disorder. These MoRFs are of high interest in drug development due to the potential to undergo a disorder-to-order transition with specific interactions or binding. Looking at the amino acid sequence of AR-NTD, there are multiple repeat regions that vary in length that include the polyproline tract (average 9 repeats), polyglycine tract (average 16 repeats), and polyglutamine tract (average 21 CAG repeats). Importantly the AR NTD has several potential MoRFs such as aromatic residues W433, Y445 and F437. An example that emphasizes the qualities that impact structure and function of intrinsically disordered proteins is the interaction of RAP74 with the AR NTD. RAP74 interacts within amino acids residues 423-446 that contain these MoRFs and has weak affinity in the millimolar range (K_D = 1749 μM) that substantially improves

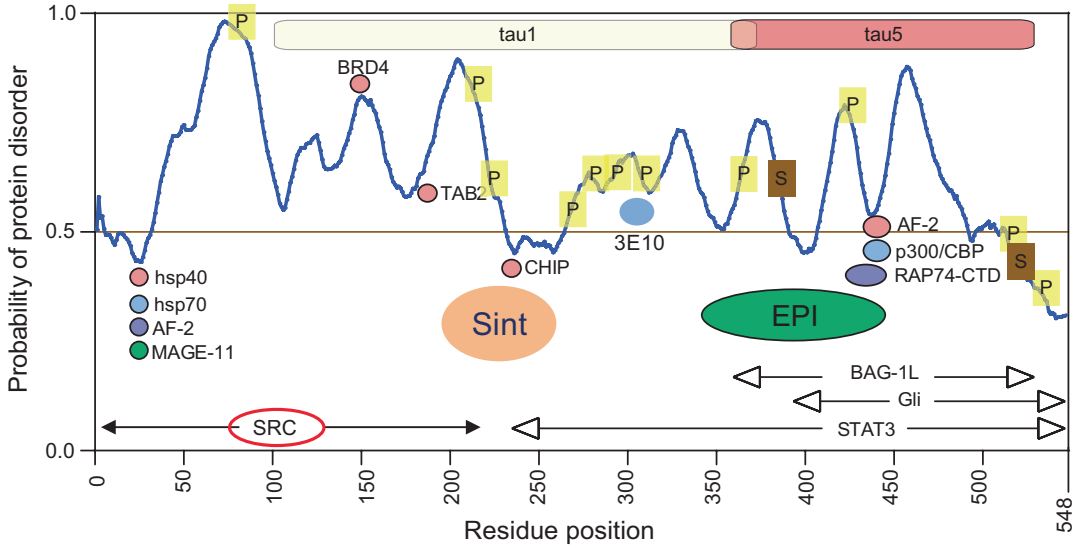

Fig. 18.3 Ronn plot of AR-NTD showing some mapped protein-protein interactions, post-translational modifications, and binding sites for small molecules that directly interact with this domain. The probability of protein disorder across the amino acid residues of the NTD. A probability score below 0.5 is considered ordered (folded) whereas a score above 0.5 is considered disordered. Transactivation units (tau) 1 and 5 are shown within the NTD. The binding sites for EPI-002, sintokamide, and 3E10-AR441 are shown. Regions of posttranslational modification and interactions with some other proteins on the N-terminal domain are shown. Phosphorylation (P) and sumoylation (S). Protein interactions shown include chaperones hsp40 and hsp90; AF-2, activation function-2 in the AR LBD for N/C interaction; RAP74 of the basal transcriptional machinery; p300/CBP, BRD4, TAB2, CHIP, MAGE-11, BAG1L, Gli, STAT3, and SRC

with phosphorylation of S424 of AR to a K_D of 702 μM [26, 109] (Fig. 18.4). Thus, an inhibitor of AR NTD even with an IC_{50} in the very high μM to millimolar range may still have therapeutic value in blocking this weak interaction with RAP74, if such blood levels are achievable without toxicity to other tissues. This difference in molecular mechanism of protein-protein interactions in the NTD compared to ligand-binding to LBD is critical to understand when considering differences between an AR NTD inhibitor and LBD inhibitors, as in the latter case an antiandrogen such as enzalutamide has to compete with the physiological ligand such as dihydrotestosterone (DHT) that has affinity in the low nM range.

The structural plasticity of the NTDs of hormone receptors allows this domain to exist as multiple and changing conformations depending on the environment and interacting partner, but also makes this domain a difficult drug target [17, 29, 35, 82]. The lack of a stable binding site together with shallow clefts for interactions with other proteins creates a challenge in drug development that is unique from the classic "lock-and-key" model for folded proteins. Intrinsically disordered proteins or regions tend to have high specificity and low affinity thereby allowing a rapid interchange of binding partners. Examples for the AR NTD are RAP74 (as described above) and Hsp70, which have binding affinities in the μM range [26, 31]. The degree of helical secondary structure of hormone receptor NTDs increases with binding to interacting proteins to conform to a molten-globule-like conformation referred to as 'collapsed disordered' [61, 71, 97]. NTD-interacting proteins that that are known to increase α-helical content include TATA-binding protein (TBP) [34, 65, 66, 116], CREB-binding protein (CBP) [61], RAP74 subunit of human transcription factor IIF [61, 97] and Jun dimerization protein 2 (JDP2) [116]. These protein-protein interactions induce α-helical structure and lead to additional protein-protein interactions to impact transcriptional activity [106, 121]. Exchange of binding partners may involve unfolding of structured regions in AF-1 with

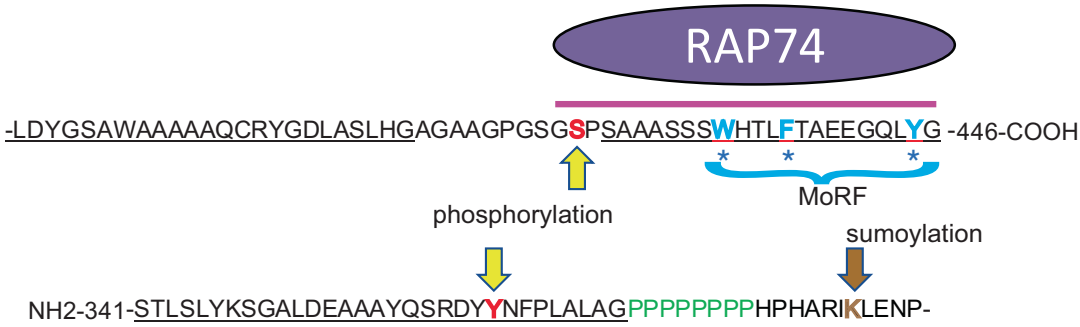

Fig. 18.4 EPI binding sites within tau5 of AR-NTD in the context of RAP74's binding site. Residues in the three EPI-002 binding sites (underlined) and the flanking residues are shown. Phosphorylation of S424 impacts the affinity of RAP74 binding to tau5. MoRFs within the RAP74 site of interaction. Other post-translational modifications that affect AR transcriptional activity and could possibly impact the binding of EPI-002 to tau5 that include Y363 that is phosphorylated by Ack and sumoylation of K386

increased structure within an adjacent region [89]. It is this plasticity that permits hormone receptors to act as a hub of interactions with an extremely large repertoire of binding partners [43, 59, 122]. Recently, low-resolution cryoelectron microscopy revealed that the structure of transcriptionally active full-length AR is unique from ERα in its direct interaction with steroid receptor coactivators (SRCs) and its orientation of dimerization [123]. AR homodimerizes in a head-to-head and tail-to-tail manner and consists of two different conformations of NTD [123]. One AR NTD conformation interacts with a single SRC-3 molecule close to its $_{23}$FQNLF$_{27}$ motif [123], consistent with earlier coimmunoprecipitation studies that showed SRC interacts within amino acid residues 1-233 of the AR-NTD [111]. Conversely, a single p300 molecule interacts with both conformations of NTD [123]. Interaction of a hormone receptor with DNA can also induce tertiary structure and α-helical content of the NTD/AF-1 to encourage protein-protein interactions with cofactors and bridging factors to ultimately impact transcriptional activity [8, 79, 80].

18.2.2 DNA-Binding Domain (DBD) and Hinge Region

The crystal structures of DBDs of hormone receptors have been resolved [105]. This domain is the most conserved in sequence compared to the other domains at greater than 75% for MR, GR, PR, and AR DBDs and 57% between ERα and AR DBDs. Hormone receptor DBDs have three α-helices that are comprised of two zinc finger motifs and a C-terminal extension (CTE). Each zinc finger has four cysteine residues that bind a zinc ion. The first zinc finger subdomain interacts with the major groove of base-specific regions of DNA and is called the P-box. The second zinc finger subdomain stabilizes receptor-DNA interaction through non-specific contacts with the DNA backbone and also contains the distal box (D box) that is involved in receptor dimerization [98]. The 22Rv1 human prostate cancer cell line is commonly used to analyze the effects of drugs on the transcriptional activity of AR including its constitutively active AR-Vs, but this cell line is unique in that its AR carries duplication of exon 3: this encodes an additional zinc finger within its DBD thereby impacting its properties such as protein half-life [108].

The CTE mediates the specificity of AR to recognize androgen response elements (AREs). The majority of AREs have been mapped to enhancers in the regulatory regions of genes regulated by androgens and consist of a repeat of two hexamers separated by a 3 base-pair spacer. It is important to note that there are general response elements that are recognized by all steroid hormone receptors with the exception of ER [112], as well those that are specific to a receptor [18, 27, 38, 54, 119]. Due to this high degree of similarity in sequence and structure across the steroid hormone receptors' DBDs, this domain

has been generally considered to be a poor drug target due to challenges to achieve specificity. Although the DBD functions to steer the receptor to specific regulatory regions of the genome, as mentioned above it also primes the NTD for interactions with specific coregulatory proteins. The genomic sequence of a particular response element can influence the transcriptional response of a gene [45].

DBDs are linked to LBDs by the hinge region, which is unstructured. The hinge region is important in the nuclear translocation of the receptor and is sequestered in the absence of ligand [93]. It contains part of the CTE involved in interactions between the DNA and receptor as well as having other functions that are regulated by post-translational modifications within this region [21, 44]. Upon binding DNA there can be a change in conformation of the CTE which stabilizes intramolecular interactions [44] and creates a binding site for coregulatory proteins [13, 95]. Hormone receptors can alter the conformation of DNA to facilitate the assembly of multi-protein complexes within the enhancer or promoter regions of target genes [44].

18.2.3 Ligand-Binding Domain (LBD)

LBDs of hormone receptors function to mediate the effects of steroids and have been the primary target for drug development. The AR-LBD is the direct or indirect target for all currently FDA-approved drugs against the androgen axis. For example, indirect drug targets for AR-LBD are those therapeutics that reduce the levels of androgen that bind to the AR-LBD and include LHRH analogues and CYP17 inhibitors (e.g. abiraterone) that block steroidogenesis. Drugs that directly target AR-LBD include both agonists that are called selective AR modifiers or "SARMs" as well as antagonists that are called, "antiandrogens". Antiandrogens can be steroidal or non-steroidal; non-steroidal antiandrogens have the stem name "lutamide" and include flutamide, nilutamide, bicalutamide, enzalutamide, apalutamide, and darolutamide. Antiandrogens are competitive inhibitors with androgens for the AR-LBD and induce an AR conformation that is not transcriptionally active. The "lutamides" have evolved since the first in class drug flutamide to be more bulky and thereby more effective in disrupting protein-protein interactions. Sequence similarity in the LBDs of hormone receptors manifests with some steroids able to bind to other receptor LBDs in addition to their cognate receptor, based upon the concentrations of steroid and also the existence of point mutations within this domain. For example, AR LBD shares 54% sequence similarity with PR-LBD and not surprisingly steroidal progestins (e.g., cyproterone acetate/6-chloro-17-hydroxy-la,2a-methylenepregna-4,6-diene-3,20-dione acetate) were the first inhibitors discovered against AR [92]. Similarly, the non-steroidal antiandrogens such bicalutamide and enzalutamide bind to PR-LBD to inhibit its transcriptional activity [10, 51].

The crystal structures of LBDs of all hormone receptors have been resolved, in complex with various ligands, with only an agonist-bound conformation available for the AR. The lack of success in obtaining a crystal structure of the AR-LBD in an antagonist conformation has impeded drug development against this important drug target. Crystal structure analyses have revealed that LBDs of hormone receptors are folded into 3 layers that form an anti-parallel α-helical sandwich with up to 12 α-helices (H1-12) and up to 4 short β-strands that may form β-sheets [14, 44, 67, 107, 120]. Hormone receptors lack helix 2 so have 11 helices, with the exception of ER that has all 12 helices [44]. Generally, the binding of an agonist induces conformational changes such that helix 12 stabilizes and covers the ligand-binding pocket to form a hydrophobic cleft and expose the AF2 region. This conformational change provides a binding interface for AF-2 to interact with LxxLL motifs of coactivators such as SRCs [14, 41, 44, 67].

In the absence of ligand, LBDs repress AF-1 transcriptional activities as shown for PR, GR and AR. For these receptors, when their LBDs are deleted the results are constitutively active receptors [8, 22, 48, 55]. Here the ER stands out from the other receptors and emphasizes that its transcriptional activity is largely through AF-2.

Deletion of ER LBD results in a 95% decrease of its transcriptional activity [68], compared to the truncated AR lacking LBD becoming constitutively activated [55]. As mentioned above, AR-LBD also dictates the contribution of transcriptional activity from tau-1 versus tau-5. For the truncated constitutively active AR-Vs that lack LBD, tau-5 would be the dominant tau driving transcriptional activity and thereby a critical drug target.

In diseases such as prostate cancer and some breast cancers, there are some structural alterations in the AR-LBD that are considered to drive the disease and confer resistance to therapies that target the AR-LBD. These structural alterations include deletion or truncation of AR LBD, resulting in constitutively active AR-Vs that are independent of androgens [55]; gain-of-function mutations in the AR-LBD underlying antiandrogen withdrawal syndromes [74]; as well as point mutations that result in promiscuous binding to other steroids [32].

18.3 Androgen Receptor

Full-length AR molecular mass is calculated as 98.9 kDa but when run on SDS-PAGE it migrates as a band of approximately 110 kDa. AR NTD has several polymorphic tracts (see above) that result in its variability in length (generally 547–556 residues), a folded DBD (65 residues), a disordered hinge region (49 residues) and folded LBD (249 residues). Full-length AR is encoded from 8 canonical exons and 7 cryptic exons in the *AR* gene. This gene resides on the X chromosome (*AR* locus: Xq11-Xq12); both males and females have only one functional copy of AR due to X-inactivation. The *AR* gene has binding sites for SP1, NFkB, and c-MYC but lacks elements for TATA and CCAAT in its regulatory region (for reviews see [15, 50]). Tissue-specific activity of AR is modulated by regulation of expression in response to androgen [50] and tissue-specific expression of its coregulators [87]. This results in tissue-specific expression of AR target genes such as prostate-specific antigen (PSA), which is a biomarker for prostate cancer.

Full-length AR mediates the effects of androgens such as testosterone and dihydrotestosterone (DHT) that are required in males for sexual differentiation, maintenance of spermatogenesis, and male gonadotropin regulation. Male reproductive tissue such as the prostate is dependent upon functional AR signaling. The dependency of prostate tissue on androgens provides the rationale for targeting full-length AR for the treatment of prostate cancer using androgen deprivation therapy (ADT) and antiandrogens. In addition to prostate cancer, the androgen axis plays a role in other pathologies such as alopecia, polycystic ovarian syndrome, spinal bulbar muscular dystrophy, androgen insensitivity syndrome, and some breast cancers, thereby emphasizing the need for therapeutic inhibitors of AR transcriptional activity (for a review see [75]).

18.4 Rationale for Developing Inhibitors to the NTD

Interest in developing drugs to the intrinsically disordered AR-NTD predominantly comes from the discovery of constitutively active AR-Vs lacking LBD that are associated with resistance mechanisms in lethal castration-resistance prostate cancer (CRPC) [4, 5, 103, 104] and have been discovered in breast cancer tissues [1, 24, 43, 49]. The fact that all of the transcriptional activity of AR resides in its NTD, also means that inhibitors of the AR-NTD would be effective against full-length AR, gain-of-function mutations in AR-LBD, and other mechanisms of maintained AR transcriptional activities. In other words, an inhibitor to the AR-NTD should block the transcriptional activities of all AR species. Also beneficial is that AR-NTD has little sequence similarity (<15%) to its most closely related hormone receptors and is thereby predicted to be a highly specific drug target.

Due to challenges in discovery of small molecules that directly bind to an intrinsically disordered target, an approach has been to target folded proteins that interact with AR-NTD. The first in vivo proof-of-concept that this could yield

a therapeutic response for CRPC was provided using decoys that sequestered AR-NTD interacting proteins [91, 96]. There have also been a number of studies that target an individual interacting binding partner of AR-NTD such as: hsp40/70 to induce degradation of AR-Vs and reduce aggregation of full-length AR with extended polyQ tracts [28, 31, 78, 88]; BRD4 [6]; BAG1L [16, 69, 73]; and SRC-1 and 3 [114] (Fig. 18.3). The approach of targeting an interacting protein rather than the AR-NTD directly has the inherent risk of lack of specificity to blocking AR function since most interacting partners are not unique for AR and interact with many other proteins. Thus, discovery of drugs that directly bind to AR-NTD is of high interest.

18.5 Small Molecule Inhibitors of AR-NTD

Currently all small molecule inhibitors proven to directly bind to the AR-NTD were originally isolated from natural compound libraries [100]. These were libraries of marine sponges that were screened to discover: sintokamides [11, 101]; naphatenones [10, 86]; and EPI-001/ralaniten [3, 90] (Fig. 18.5). Of these three unique chemical scaffolds, the EPI-001/ralaniten analogues were the first drugs against AR-NTD to reach human clinical trials. Importantly, ralaniten was also the first drug that directly binds to any intrinsically disordered target to reach clinical testing, marking a breakthrough in drug discovery for intrinsically disordered proteins. Recently, a second-generation analogue of ralaniten began clinical trials in heavily pretreated men with CRPC (NCT04421222) and in combination with enzalutamide ([46]; NCT05075577). Combinations of EPI compounds have shown improved therapeutic responses for CRPC when combined with antiandrogens [46], radiation [9], PIN1 inhibitors [76], palbociclib [110], mTOR inhibitors [58], taxanes [83], and sintokamides [11]. The sintokamides are still under development for the clinic as potential imaging agents and to use in combination with tau5 inhibitors. Drug development for naphatenones, that were first isolated from the marine sponge Niphates digitalis, was stopped due to their reactivity and alkylation of glutathione [10, 86].

Sintokamides A to E were isolated from the marine sponge Dysidea sp. These were the first small molecules that inhibited the AR-NTD to be published [101]. Sintokamide A (SINT1) directly binds AR AF-1 region to specifically inhibit transactivation of AR NTD and block transcriptional activities of full-length AR and AR-Vs [11]. In vivo studies with sintokamide using human prostate cancer xenografts grown in castrated mice revealed regression of tumours and reduced expression of the AR-regulated gene, PSA [11]. Interestingly, additive inhibition was evident when SINT1 was combined with ralaniten, which suggested SINT1 binds to a site on AF-1 that is unique from that bound by ralaniten [11]. Through studying well-characterized protein-protein interactions with the AR-NTD, differences in blocking interaction of STAT3 with AR-NTD between these compounds revealed that SINT1 probably interacts more N-terminally within tau-1 whereas ralaniten interacts with tau-5 [11, 25]. The inability of the sintokamides to impact IL-6 transactivation of AR and STAT3 interaction with AR-NTD predicts that these compounds would be ineffective against prostatic bone lesions that have elevated levels of IL-6 and are prevalent in men with advanced prostate cancer.

The first EPI compound, EPI-067, was isolated from the marine sponge Geodia lindgreni [2, 99, 100]. Structure activity relationship studies of several hundred analogues yielded EPI-002, a single stereoisomer of the mixture called EPI-001, that was developed for first-in-human clinical trials. These compounds have a chlorohydrin and consistent with the literature were demonstrated to not be reactive as shown at physiological pH in vitro [11] and in vivo using a radioactive imaging agent [51], as well as from patient clinical samples [94, 113]. EPI-002 was established as a first-in-class compound called ralaniten by the USAN Council with a stem name of “aniten” based upon its unique mechanism of action that distinguishes these compounds from the “lutamide” antiandrogens such as enzalu-

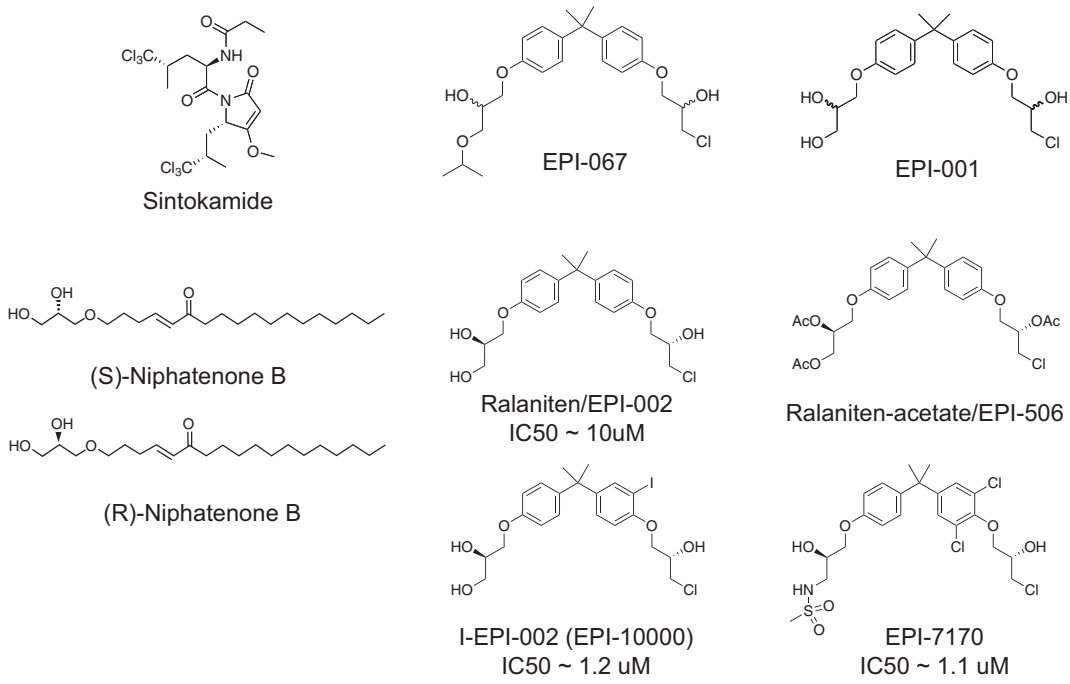

Fig. 18.5 Chemical structures of small molecules validated to directly bind to AF-1 within the AR-NTD. Sintokamides do not block IL-6 transactivation of the AR-NTD or STAT-3 interaction with AR-NTD so are being developed as imaging agents rather than as therapeutics. Niphatenones were reactive and formed glutathione adducts and have been dropped from clinical development. EPI-compounds showing the discovery compound, EPI-067, isolated from a marine sponge [2]. EPI-001 is a mixture of 4 stereoisomers including the active compound ralaniten (EPI-002) which was delivered as a prodrug (ralaniten-acetate or EPI-506) to prostate cancer patients [100]. Addition of a halogen (iodine) to a phenyl ring, I-EPI-002 (EPI-10000), improved the potency by 10-fold compared to EPI-002 [51]. Removal of the primary alcohol as with the second-generation compound EPI-7170 improves the in vivo efficacy presumably due to reduced metabolism [9, 94]

tamide. Ralaniten predominantly binds residues 341-446 of tau-5, including the core unit $_{435}$WHTLF$_{439}$, plus some overlap into tau-1 (101-370) [25] (Figs. 18.3 and 18.4). As predicted, ralaniten inhibits the transcriptional activities of full-length AR, AR-Vs, gain-of-function AR-LBD mutations, AR with altered polyQ tracts, and AR-transcriptional activities with aberrant expression of coactivators and amplified levels of AR [3, 90, 121]. Inhibition of AR transcriptional activity by EPI was specific, with no impact on related human hormone receptors. EPI analogues inhibited AR-NTD interaction with CREB-binding protein (CBP) and RAP74 [3]. They do not induce AR nuclear translocation in the absence of androgen [3]. Importantly EPI analogues block AR DNA binding in the promoters and enhancers of target genes to decrease expression of these genes in response to androgens [3, 90]. In vivo efficacy of EPI as a therapeutic for prostate cancer was demonstrated using xenografts of human prostate cancer cell lines, patient-derived xenografts, and the Herschberger assay [3].

The technical hurdle of aggregation of recombinant intrinsically disordered proteins makes it difficult to provide evidence of direct binding using cell-free assays. In spite of this, evidence of direct interaction of the EPI analogues with the AF-1 region of AR was shown by application of recombinant AF-1 protein in a cell-free assay by fluorescence emission spectroscopy [3] and Click-chemistry probes [90]. Importantly, cell-free assays may be prone to producing artifacts because of the sensitivity of the conformation(s) of an intrinsically disordered protein on its envi-

ronment and protein-protein interactions. The first evidence of direct binding for any intrinsically disordered protein in cells was provided when EPI bound the endogenous AR in LNCaP human prostate cancer cells. A number of approaches were employed that included both click-chemistry probes and radiolabelled analogues [51, 90]. In vivo data from castrated mice injected with a radiolabelled EPI analogue also provided strong evidence of specificity of EPI compounds for the AR as well as proof-of-concept of the potential of these compounds to image tumours that express AR-NTD [51]. Later, Dr. Salvatella and his team provided NMR data confirming that EPI compounds bind specifically to AF-1 within tau-5 and identified the amino acid residues required for this interaction [25]. There were three regions within AR AF-1 that were required for EPI to bind, implying that EPI binds within a pocket rather than to linear amino acid sequence (Fig. 18.4). Of note, the EPI-binding site on AF-1 is also where RAP74 interacts [26] thereby supporting earlier studies showing EPI blocked this interaction [3]. Post-translational modifications within this region that may alter the binding of EPI include phosphorylation of Y363 and S424 as well as sumoylation of K386 within the flanking region. Studies to address the impact of post-translational modifications on EPI binding to AR-NTD will be important to predict potential resistance mechanisms.

18.6 First-in-Human Clinical Trials

In November 2015, the prodrug of ralaniten, called ralaniten-acetate and also known as EPI-506, was administered to the first CRPC patient enrolled in first-in-human clinical trials (NCT02606123). This marked an important event of ralaniten being the first drug that directly binds to any intrinsically disordered protein to reach a clinical trial - in oncology and even more notably in any disease. This Phase I clinical trial was a dose-escalation study in 28 heavily pretreated CRPC patients in whom abiraterone and/or enzalutamide had previously failed. The drug did show signs of efficacy in some patients as evidenced by a reduction of serum PSA and stable disease, especially in those patients receiving higher doses. A few patients remained on ralaniten for more than 1 year with stable disease. These indications of efficacy were in spite of patients having steady-state C_{min} blood concentrations that were 50× lower than what would be optimal based upon in vitro data of 25 μM [100]. Notably, the most highly dosed patients who received 3600 mg/daily had blood trough levels of only 200 ng/mL which is equivalent to 0.5 μM. These blood levels are also 48- to 58-fold lower than steady-state C_{min} for enzalutamide and its active metabolite respectively [53]. This extremely poor pharmacokinetic profile for ralaniten resulted in excessive pill burden and ultimately stopping its clinical development, in spite of it being well-tolerated. Subsequent analyses of samples from these patients revealed that ralaniten was oxidized and glucuronidated predominantly at the alcohol groups [94]. A second generation set of analogues have been designed to improve the metabolic stability of this class of drugs and these include EPI-7170 [9] and the clinical compound EPI-7386, which entered clinical trials in June 2020 for men with metastatic CRPC (NCT04421222). Early data released at ASCO-GU in February 2021 stated, "Despite the suboptimal 200 mg dose, one out of three patients who completed 12 weeks of therapy experienced a prostate specific antigen ("PSA") decline of more than 50% after three cycles of EPI-7386 therapy (12 weeks) with ongoing continued PSA declines continuing through six cycles of therapy, despite previously having failed enzalutamide and abiraterone acetate" [72, 77].

18.7 Conclusions

It is estimated that 33–50% of the proteome is intrinsically disordered or has intrinsically disordered regions [115]. The plasticity of intrinsically disordered proteins allows for multiple and changing conformations to enable the exchange of numerous binding partners. Proteins that possess intrinsic disorder tend to have functions within signaling networks and include transcrip-

tion factors such as nuclear hormone receptors and regulators of the cell cycle. Thus, it is not surprising that intrinsically disordered proteins are associated with many diseases such as cancer, diabetes, cardiovascular disease, and amyloidosis (for a review article see [60]) and are a rich potential source of drug targets. Progress on developing inhibitors that directly bind to the intrinsically disordered AR NTD, that are the first to reach clinical trials in humans for this class of proteins, may help lead future successes against other intrinsically disordered drug targets.

References

1. Aceto N, Bardia A, Wittner BS, Donaldson MC, O'Keefe R, Engstrom A, Bersani F, Zheng Y, Comaills V, Niederhoffer K, Zhu H, Mackenzie O, Shioda T, Sgroi D, Kapur R, Ting DT, Moy B, Ramaswamy S, Toner M, Haber DA, Maheswaran S (2018) AR expression in breast cancer CTCs associates with bone metastases. Mol Cancer Res 16:720–727
2. Andersen RJ (2017) Sponging off nature for new drug leads. Biochem Pharmacol 139:3–14
3. Andersen RJ, Mawji NR, Wang J, Wang G, Haile S, Myung JK, Watt K, Tam T, Yang YC, Banuelos CA, Williams DE, Mcewan IJ, Wang Y, Sadar MD (2010) Regression of castrate-recurrent prostate cancer by a small-molecule inhibitor of the amino-terminus domain of the androgen receptor. Cancer Cell 17:535–546
4. Antonarakis ES, Lu C, Wang H, Luber B, Nakazawa M, Roeser JC, Chen Y, Mohammad TA, Chen Y, Fedor HL, Lotan TL, Zheng Q, De Marzo AM, Isaacs JT, Isaacs WB, Nadal R, Paller CJ, Denmeade SR, Carducci MA, Eisenberger MA, Luo J (2014) AR-V7 and resistance to enzalutamide and abiraterone in prostate cancer. N Engl J Med 371:1028–1038
5. Armstrong AJ, Halabi S, Luo J, Nanus DM, Giannakakou P, Szmulewitz RZ, Danila DC, Healy P, Anand M, Rothwell CJ, Rasmussen J, Thornburg B, Berry WR, Wilder RS, Lu C, Chen Y, Silberstein JL, Kemeny G, Galletti G, Somarelli JA, Gupta S, Gregory SG, Scher HI, Dittamore R, Tagawa ST, Antonarakis ES, George DJ (2019) Prospective multicenter validation of androgen receptor splice variant 7 and hormone therapy resistance in high-risk castration-resistant prostate cancer: the Prophecy study. J Clin Oncol 37:1120–1129
6. Asangani IA, Dommeti VL, Wang X, Malik R, Cieslik M, Yang R, Escara-Wilke J, Wilder-Romans K, Dhanireddy S, Engelke C, Iyer MK, Jing X, Wu YM, Cao X, Qin ZS, Wang S, Feng FY, Chinnaiyan AM (2014) Therapeutic targeting of BET bromodomain proteins in castration-resistant prostate cancer. Nature 510:278–282
7. Bah A, Forman-Kay JD (2016) Modulation of intrinsically disordered protein function by post-translational modifications. J Biol Chem 291:6696–6705
8. Bain DL, Franden MA, Mcmanaman JL, Takimoto GS, Horwitz KB (2000) The N-terminal region of the human progesterone A-receptor. Structural analysis and the influence of the DNA binding domain. J Biol Chem 275:7313–7320
9. Banuelos CA, Ito Y, Obst JK, Mawji NR, Wang J, Hirayama Y, Leung JK, Tam T, Tien AH, Andersen RJ, Sadar MD (2020) Ralaniten sensitizes enzalutamide-resistant prostate cancer to ionizing radiation in prostate cancer cells that express androgen receptor splice variants. Cancers (Basel) 12:1991
10. Banuelos CA, Lal A, Tien AH, Shah N, Yang YC, Mawji NR, Meimetis LG, Park J, Kunzhong J, Andersen RJ, Sadar MD (2014) Characterization of niphatenones that inhibit androgen receptor N-terminal domain. PLoS One 9:e107991
11. Banuelos CA, Tavakoli I, Tien AH, Caley DP, Mawji NR, Li Z, Wang J, Yang YC, Imamura Y, Yan L, Wen JG, Andersen RJ, Sadar MD (2016) Sintokamide A is a novel antagonist of androgen receptor that uniquely binds activation function-1 in its amino-terminal domain. J Biol Chem 291:22231–22243
12. Beato M (1989) Gene regulation by steroid hormones. Cell 56:335–344
13. Blanco JC, Minucci S, Lu J, Yang XJ, Walker KK, Chen H, Evans RM, Nakatani Y, Ozato K (1998) The histone acetylase PCAF is a nuclear receptor coactivator. Genes Dev 12:1638–1651
14. Bourguet W, Germain P, Gronemeyer H (2000) Nuclear receptor ligand-binding domains: three-dimensional structures, molecular interactions and pharmacological implications. Trends Pharmacol Sci 21:381–388
15. Burnstein KL (2005) Regulation of androgen receptor levels: implications for prostate cancer progression and therapy. J Cell Biochem 95:657–669
16. Cato L, Neeb A, Sharp A, Buzon V, Ficarro SB, Yang L, Muhle-Goll C, Kuznik NC, Riisnaes R, Nava Rodrigues D, Armant O, Gourain V, Adelmant G, Ntim EA, Westerling T, Dolling D, Rescigno P, Figueiredo I, Fauser F, Wu J, Rottenberg JT, Shatkina L, Ester C, Luy B, Puchta H, Troppmair J, Jung N, Brase S, Strahle U, Marto JA, Nienhaus GU, Al-Lazikani B, Salvatella X, De Bono JS, Cato AC, Brown M (2017) Development of Bag-1L as a therapeutic target in androgen receptor-dependent prostate cancer. elife 6:e27159
17. Choi UB, Sanabria H, Smirnova T, Bowen ME, Weninger KR (2019) Spontaneous switching among conformational ensembles in intrinsically disordered proteins. Biomolecules 9:114
18. Claessens F, Alen P, Devos A, Peeters B, Verhoeven G, Rombauts W (1996) The androgen-specific pro-

basin response element 2 interacts differentially with androgen and glucocorticoid receptors. J Biol Chem 271:19013–19016
19. Claessens F, Joniau S, Helsen C (2017) Comparing the rules of engagement of androgen and glucocorticoid receptors. Cell Mol Life Sci 74:2217–2228
20. Cleutjens CB, Steketee K, Van Eekelen CC, Van Der Korput JA, Brinkmann AO, Trapman J (1997) Both androgen receptor and glucocorticoid receptor are able to induce prostate-specific antigen expression, but differ in their growth-stimulating properties of LNCaP cells. Endocrinology 138:5293–5300
21. Clinckemalie L, Vanderschueren D, Boonen S, Claessens F (2012) The hinge region in androgen receptor control. Mol Cell Endocrinol 358:1–8
22. Danielsen M, Northrop JP, Jonklaas J, Ringold GM (1987) Domains of the glucocorticoid receptor involved in specific and nonspecific deoxyribonucleic acid binding, hormone activation, and transcriptional enhancement. Mol Endocrinol 1:816–822
23. Darling AL, Uversky VN (2018) Intrinsic disorder and posttranslational modifications: the darker side of the biological dark matter. Front Genet 9:158
24. De Kruijff IE, Sieuwerts AM, Onstenk W, Jager A, Hamberg P, De Jongh FE, Smid M, Kraan J, Timmermans MA, Martens JWM, Sleijfer S (2019) Androgen receptor expression in circulating tumor cells of patients with metastatic breast cancer. Int J Cancer 145:1083–1089
25. De Mol E, Fenwick RB, Phang CT, Buzon V, Szulc E, De La Fuente A, Escobedo A, Garcia J, Bertoncini CW, Estebanez-Perpina E, Mcewan IJ, Riera A, Salvatella X (2016) EPI-001, a compound active against castration-resistant prostate cancer, targets transactivation unit 5 of the androgen receptor. ACS Chem Biol 11:2499–2505
26. De Mol E, Szulc E, Di Sanza C, Martinez-Cristobal P, Bertoncini CW, Fenwick RB, Frigole-Vivas M, Masin M, Hunter I, Buzon V, Brun-Heath I, Garcia J, De Fabritiis G, Estebanez-Perpina E, Mcewan IJ, Nebreda AR, Salvatella X (2018) Regulation of androgen receptor activity by transient interactions of its transactivation domain with general transcription regulators. Structure 26:145–152 e3
27. Devos A, Claessens F, Alen P, Winderickx J, Heyns W, Rombauts W, Peeters B (1997) Identification of a functional androgen-response element in the exon 1-coding sequence of the cystatin-related protein gene crp2. Mol Endocrinol 11:1033–1043
28. Dong J, Wu Z, Wang D, Pascal LE, Nelson JB, Wipf P, Wang Z (2019) Hsp70 binds to the androgen receptor N-terminal domain and modulates the receptor function in prostate cancer cells. Mol Cancer Ther 18:39–50
29. Dunker AK, Brown CJ, Lawson JD, Iakoucheva LM, Obradovic Z (2002) Intrinsic disorder and protein function. Biochemistry 41:6573–6582
30. Dyson HJ, Wright PE (2005) Intrinsically unstructured proteins and their functions. Nat Rev Mol Cell Biol 6:197–208
31. Eftekharzadeh B, Banduseela VC, Chiesa G, Martinez-Cristobal P, Rauch JN, Nath SR, Schwarz DMC, Shao H, Marin-Argany M, Di Sanza C, Giorgetti E, Yu Z, Pierattelli R, Felli IC, Brun-Heath I, Garcia J, Nebreda AR, Gestwicki JE, Lieberman AP, Salvatella X (2019) Hsp70 and Hsp40 inhibit an inter-domain interaction necessary for transcriptional activity in the androgen receptor. Nat Commun 10:3562
32. Eisermann K, Wang D, Jing Y, Pascal LE, Wang Z (2013) Androgen receptor gene mutation, rearrangement, polymorphism. Transl Androl Urol 2:137–147
33. Evans RM (1988) The steroid and thyroid hormone receptor superfamily. Science 240:889–895
34. Fischer K, Kelly SM, Watt K, Price NC, Mcewan IJ (2010) Conformation of the mineralocorticoid receptor N-terminal domain: evidence for induced and stable structure. Mol Endocrinol 24:1935–1948
35. Fisher CK, Stultz CM (2011) Constructing ensembles for intrinsically disordered proteins. Curr Opin Struct Biol 21:426–431
36. Gelmann EP (2002) Molecular biology of the androgen receptor. J Clin Oncol 20:3001–3015
37. Goicochea NL, Garnovskaya M, Blanton MG, Chan G, Weisbart R, Lilly MB (2017) Development of cell-penetrating bispecific antibodies targeting the N-terminal domain of androgen receptor for prostate cancer therapy. Protein Eng Des Sel 30:785–793
38. Green S, Kumar V, Theulaz I, Wahli W, Chambon P (1988a) The N-terminal DNA-binding 'zinc finger' of the oestrogen and glucocorticoid receptors determines target gene specificity. EMBO J 7:3037–3044
39. Griekspoor A, Zwart W, Neefjes J, Michalides R (2007) Visualizing the action of steroid hormone receptors in living cells. Nucl Recept Signal 5:e003
40. He Y, Chen Y, Mooney SM, Rajagopalan K, Bhargava A, Sacho E, Weninger K, Bryan PN, Kulkarni P, Orban J (2015) Phosphorylation-induced conformational ensemble switching in an intrinsically disordered cancer/testis antigen. J Biol Chem 290:25090–25102
41. Heery DM, Kalkhoven E, Hoare S, Parker MG (1997) A signature motif in transcriptional coactivators mediates binding to nuclear receptors. Nature 387:733–736
42. Hermanson O, Glass CK, Rosenfeld MG (2002) Nuclear receptor coregulators: multiple modes of modification. Trends Endocrinol Metab 13:55–60
43. Hickey TE, Irvine CM, Dvinge H, Tarulli GA, Hanson AR, Ryan NK, Pickering MA, Birrell SN, Hu DG, Mackenzie PI, Russell R, Caldas C, Raj GV, Dehm SM, Plymate SR, Bradley RK, Tilley WD, Selth LA (2015) Expression of androgen receptor splice variants in clinical breast cancers. Oncotarget 6:44728–44744
44. Hill KK, Roemer SC, Churchill ME, Edwards DP (2012) Structural and functional analysis of domains of the progesterone receptor. Mol Cell Endocrinol 348:418–429

45. Hilser VJ, Thompson EB (2011) Structural dynamics, intrinsic disorder, and allostery in nuclear receptors as transcription factors. J Biol Chem 286:39675–39682
46. Hirayama Y, Tam T, Jian K, Andersen RJ, Sadar MD (2020) Combination therapy with androgen receptor N-terminal domain antagonist EPI-7170 and enzalutamide yields synergistic activity in AR-V7-positive prostate cancer. Mol Oncol 14:2455–2470
47. Holden NS, George T, Rider CF, Chandrasekhar A, Shah S, Kaur M, Johnson M, Siderovski DP, Leigh R, Giembycz MA, Newton R (2014) Induction of regulator of G-protein signaling 2 expression by long-acting beta2-adrenoceptor agonists and glucocorticoids in human airway epithelial cells. J Pharmacol Exp Ther 348:12–24
48. Hollenberg SM, Giguere V, Segui P, Evans RM (1987) Colocalization of DNA-binding and transcriptional activation functions in the human glucocorticoid receptor. Cell 49:39–46
49. Hu DG, Hickey TE, Irvine C, Wijayakumara DD, Lu L, Tilley WD, Selth LA, Mackenzie PI (2014) Identification of androgen receptor splice variant transcripts in breast cancer cell lines and human tissues. Horm Cancer 5:61–71
50. Hunter I, Hay CW, Esswein B, Watt K, Mcewan IJ (2018) Tissue control of androgen action: the ups and downs of androgen receptor expression. Mol Cell Endocrinol 465:27–35
51. Imamura Y, Tien AH, Pan J, Leung JK, Banuelos CA, Jian K, Wang J, Mawji NR, Fernandez JG, Lin KS, Andersen RJ, Sadar MD (2016) An imaging agent to detect androgen receptor and its active splice variants in prostate cancer. JCI Insight 1:e87850
52. Isikbay M, Otto K, Kregel S, Kach J, Cai Y, Vander Griend DJ, Conzen SD, Szmulewitz RZ (2014) Glucocorticoid receptor activity contributes to resistance to androgen-targeted therapy in prostate cancer. Horm Cancer 5:72–89
53. Ito Y, Sadar MD (2018) Enzalutamide and blocking androgen receptor in advanced prostate cancer: lessons learnt from the history of drug development of antiandrogens. Res Rep Urol 10:23–32
54. Jantzen HM, Strahle U, Gloss B, Stewart F, Schmid W, Boshart M, Miksicek R, Schutz G (1987) Cooperativity of glucocorticoid response elements located far upstream of the tyrosine aminotransferase gene. Cell 49:29–38
55. Jenster G, Van Der Korput HA, Trapman J, Brinkmann AO (1995) Identification of two transcription activation units in the N-terminal domain of the human androgen receptor. J Biol Chem 270:7341–7346
56. Jenster G, Van Der Korput HA, Van Vroonhoven C, Van Der Kwast TH, Trapman J, Brinkmann AO (1991) Domains of the human androgen receptor involved in steroid binding, transcriptional activation, and subcellular localization. Mol Endocrinol 5:1396–1404
57. Johnson AB, O'Malley BW (2012) Steroid receptor coactivators 1, 2, and 3: critical regulators of nuclear receptor activity and steroid receptor modulator (SRM)-based cancer therapy. Mol Cell Endocrinol 348:430–439
58. Kato M, Banuelos CA, Imamura Y, Leung JK, Caley DP, Wang J, Mawji NR, Sadar MD (2016) Cotargeting androgen receptor splice variants and mTOR signaling pathway for the treatment of castration-resistant prostate cancer. Clin Cancer Res 22:2744–2754
59. Krasowski MD, Reschly EJ, Ekins S (2008) Intrinsic disorder in nuclear hormone receptors. J Proteome Res 7:4359–4372
60. Kulkarni P, Uversky VN (2019) Intrinsically disordered proteins in chronic diseases. Biomolecules 9:147
61. Kumar R, Betney R, Li J, Thompson EB, Mcewan IJ (2004a) Induced alpha-helix structure in AF1 of the androgen receptor upon binding transcription factor TFIIF. Biochemistry 43:3008–3013
62. Kumar R, Moure CM, Khan SH, Callaway C, Grimm SL, Goswami D, Griffin PR, Edwards DP (2013) Regulation of the structurally dynamic N-terminal domain of progesterone receptor by protein-induced folding. J Biol Chem 288:30285–30299
63. Kumar R, Thompson EB (1999) The structure of the nuclear hormone receptors. Steroids 64:310–319
64. Kumar R, Thompson EB (2003) Transactivation functions of the N-terminal domains of nuclear hormone receptors: protein folding and coactivator interactions. Mol Endocrinol 17:1–10
65. Kumar R, Thompson EB (2019) Role of phosphorylation in the modulation of the glucocorticoid receptor's intrinsically disordered domain. Biomolecules 9:95
66. Kumar R, Volk DE, Li J, Lee JC, Gorenstein DG, Thompson EB (2004b) TATA box binding protein induces structure in the recombinant glucocorticoid receptor AF1 domain. Proc Natl Acad Sci U S A 101:16425–16430
67. Kumar R, Zakharov MN, Khan SH, Miki R, Jang H, Toraldo G, Singh R, Bhasin S, Jasuja R (2011) The dynamic structure of the estrogen receptor. J Amino Acids 2011:812540
68. Kumar V, Green S, Stack G, Berry M, Jin JR, Chambon P (1987) Functional domains of the human estrogen receptor. Cell 51:941–951
69. Kuznik NC, Solozobova V, Jung N, Grassle S, Lei Q, Lewandowski EM, Munuganti R, Zoubeidi A, Chen Y, Brase S, Cato ACB (2021) Development of a benzothiazole scaffold-based androgen receptor N-terminal inhibitor for treating androgen-responsive prostate cancer. ACS Chem Biol 16:2103–2108
70. Laudet V, Hanni C, Coll J, Catzeflis F, Stehelin D (1992) Evolution of the nuclear receptor gene superfamily. EMBO J 11:1003–1013
71. Lavery DN, Mcewan IJ (2008) Structural characterization of the native NH2-terminal transactivation

domain of the human androgen receptor: a collapsed disordered conformation underlies structural plasticity and protein-induced folding. Biochemistry 47:3360–3369
72. Le Moigne R, Pearson P, Lauriault V, Hong NH, Virsik P, Zhou HJ, Cesano A (2021) Preclinical and clinical pharmacology of EPI-7386, an androgen receptor N-terminal domain inhibitor for castration-resistant prostate cancer. J Clin Oncol 39:119
73. Lee I, Kuznik NC, Rottenberg JT, Brown M, Cato ACB (2019) BAG1L: a promising therapeutic target for androgen receptor-dependent prostate cancer. J Mol Endocrinol 62:R289–R299
74. Leone G, Tucci M, Buttigliero C, Zichi C, Pignataro D, Bironzo P, Vignani F, Scagliotti GV, Di Maio M (2018) Antiandrogen withdrawal syndrome (AAWS) in the treatment of patients with prostate cancer. Endocr Relat Cancer 25:R1–R9
75. Leung JK, Tien AH, Sadar MD (2021a) Androgen receptors in the pathology of disease. In: Badr MZ (ed) Nuclear receptors: the art and science of modulator design and discovery. Springer, Cham
76. Leung JK, Imamura Y, Kato M, Wang J, Mawji NR, Sadar MD (2021b) Pin1 inhibition improves the efficacy of ralaniten compounds that bind to the N-terminal domain of androgen receptor. Commun Biol 4:v381
77. Lifesciences (2021) ESSA pharma presents favorable initial phase 1 clinical pharmacology data of EPI-7386 for advanced forms of prostate cancer at the 2021 ASCO genitourinary cancers symposium [Online]. Available: https://lifesciencesbc.ca/members/essa-pharma-presents-favorable-initial-phase-1-clinical-pharmacology-data-of-epi-7386-for-advanced-forms-of-prostate-cancer-at-the-2021-asco-genitourinary-cancers-symposium [Accessed]
78. Liu C, Lou W, Yang JC, Liu L, Armstrong CM, Lombard AP, Zhao R, Noel ODV, Tepper CG, Chen HW, Dall'Era M, Evans CP, Gao AC (2018) Proteostasis by STUB1/HSP70 complex controls sensitivity to androgen receptor targeted therapy in advanced prostate cancer. Nat Commun 9:4700
79. Loven MA, Davis RE, Curtis CD, Muster N, Yates JR, Nardulli AM (2004) A novel estrogen receptor alpha-associated protein alters receptor-deoxyribonucleic acid interactions and represses receptor-mediated transcription. Mol Endocrinol 18:2649–2659
80. Loven MA, Likhite VS, Choi I, Nardulli AM (2001) Estrogen response elements alter coactivator recruitment through allosteric modulation of estrogen receptor beta conformation. J Biol Chem 276:45282–45288
81. Mangelsdorf DJ, Thummel C, Beato M, Herrlich P, Schutz G, Umesono K, Blumberg B, Kastner P, Mark M, Chambon P, Evans RM (1995) The nuclear receptor superfamily: the second decade. Cell 83:835–839
82. Marsh JA, Forman-Kay JD (2012) Ensemble modeling of protein disordered states: experimental restraint contributions and validation. Proteins 80:556–572
83. Martin SK, Banuelos CA, Sadar MD, Kyprianou N (2014) N-terminal targeting of androgen receptor variant enhances response of castration resistant prostate cancer to taxane chemotherapy. Mol Oncol 9:628–639
84. Matias PM, Donner P, Coelho R, Thomaz M, Peixoto C, Macedo S, Otto N, Joschko S, Scholz P, Wegg A, Basler S, Schafer M, Egner U, Carrondo MA (2000) Structural evidence for ligand specificity in the binding domain of the human androgen receptor. Implications for pathogenic gene mutations. J Biol Chem 275:26164–26171
85. Mcinerney EM, Katzenellenbogen BS (1996) Different regions in activation function-1 of the human estrogen receptor required for antiestrogen- and estradiol-dependent transcription activation. J Biol Chem 271:24172–24178
86. Meimetis LG, Williams DE, Mawji NR, Banuelos CA, Lal AA, Park JJ, Tien AH, Fernandez JG, De Voogd NJ, Sadar MD, Andersen RJ (2012) Niphatenones, glycerol ethers from the sponge Niphates digitalis block androgen receptor transcriptional activity in prostate cancer cells: structure elucidation, synthesis, and biological activity. J Med Chem 55:503–514
87. Miller CP, Shomali M, Lyttle CR, O'Dea LS, Herendeen H, Gallacher K, Paquin D, Compton DR, Sahoo B, Kerrigan SA, Burge MS, Nickels M, Green JL, Katzenellenbogen JA, Tchesnokov A, Hattersley G (2011) Design, synthesis, and preclinical characterization of the selective androgen receptor modulator (SARM) RAD140. ACS Med Chem Lett 2:124–129
88. Moses MA, Kim YS, Rivera-Marquez GM, Oshima N, Watson MJ, Beebe KE, Wells C, Lee S, Zuehlke AD, Shao H, Bingman WE 3rd, Kumar V, Malhotra SV, Weigel NL, Gestwicki JE, Trepel JB, Neckers LM (2018) Targeting the Hsp40/Hsp70 chaperone axis as a novel strategy to treat castration-resistant prostate cancer. Cancer Res 78:4022–4035
89. Motlagh HN, Hilser VJ (2012) Agonism/antagonism switching in allosteric ensembles. Proc Natl Acad Sci U S A 109:4134–4139
90. Myung JK, Banuelos CA, Fernandez JG, Mawji NR, Wang J, Tien AH, Yang YC, Tavakoli I, Haile S, Watt K, Mcewan IJ, Plymate S, Andersen RJ, Sadar MD (2013) An androgen receptor N-terminal domain antagonist for treating prostate cancer. J Clin Invest 123:2948–2960
91. Myung JK, Wang G, Chiu HH, Wang J, Mawji NR, Sadar MD (2017) Inhibition of androgen receptor by decoy molecules delays progression to castration-recurrent prostate cancer. PLoS One 12:e0174134
92. Neumann F, Elger W (1966) The effect of a new antiandrogenic steroid, 6-chloro-17-Hydroxy-1alpha, 2alpha-methylenepregna-4,6-diene-3,20-dione acetate (cyproterone acetate) on the sebaceous glands of mice. J Invest Dermatol 46:561–572

93. Ni L, Llewellyn R, Kesler CT, Kelley JB, Spencer A, Snow CJ, Shank L, Paschal BM (2013) Androgen induces a switch from cytoplasmic retention to nuclear import of the androgen receptor. Mol Cell Biol 33:4766–4778
94. Obst JK, Wang J, Jian K, Williams DE, Tien AH, Mawji N, Tam T, Yang YC, Andersen RJ, Chi KN, Montgomery B, Sadar MD (2019) Revealing metabolic liabilities of ralaniten to enhance novel androgen receptor targeted therapies. ACS Pharmacol Transl Sci 2:453–467
95. Poukka H, Karvonen U, Janne OA, Palvimo JJ (2000) Covalent modification of the androgen receptor by small ubiquitin-like modifier 1 (SUMO-1). Proc Natl Acad Sci U S A 97:14145–14150
96. Quayle SN, Mawji NR, Wang J, Sadar MD (2007) Androgen receptor decoy molecules block the growth of prostate cancer. Proc Natl Acad Sci U S A 104:1331–1336
97. Reid J, Kelly SM, Watt K, Price NC, Mcewan IJ (2002) Conformational analysis of the androgen receptor amino-terminal domain involved in transactivation. Influence of structure-stabilizing solutes and protein-protein interactions. J Biol Chem 277:20079–20086
98. Roemer SC, Donham DC, Sherman L, Pon VH, Edwards DP, Churchill ME (2006) Structure of the progesterone receptor-deoxyribonucleic acid complex: novel interactions required for binding to half-site response elements. Mol Endocrinol 20:3042–3052
99. Sadar MD (2011) Small molecule inhibitors targeting the "achilles' heel" of androgen receptor activity. Cancer Res 71:1208–1213
100. Sadar MD (2020) Discovery of drugs that directly target the intrinsically disordered region of the androgen receptor. Expert Opin Drug Discovery 15:551–560
101. Sadar MD, Williams DE, Mawji NR, Patrick BO, Wikanta T, Chasanah E, Irianto HE, Soest RV, Andersen RJ (2008) Sintokamides A to E, chlorinated peptides from the sponge Dysidea sp. that inhibit transactivation of the N-terminus of the androgen receptor in prostate cancer cells. Org Lett 10:4947–4950
102. Sahu B, Laakso M, Pihlajamaa P, Ovaska K, Sinielnikov I, Hautaniemi S, Janne OA (2013) FoxA1 specifies unique androgen and glucocorticoid receptor binding events in prostate cancer cells. Cancer Res 73:1570–1580
103. Scher HI, Graf RP, Schreiber NA, Jayaram A, Winquist E, Mclaughlin B, Lu D, Fleisher M, Orr S, Lowes L, Anderson A, Wang Y, Dittamore R, Allan AL, Attard G, Heller G (2018) Assessment of the validity of nuclear-localized androgen receptor splice variant 7 in circulating tumor cells as a predictive biomarker for castration-resistant prostate cancer. JAMA Oncol 4:1179–1186
104. Scher HI, Lu D, Schreiber NA, Louw J, Graf RP, Vargas HA, Johnson A, Jendrisak A, Bambury R, Danila D, Mclaughlin B, Wahl J, Greene SB, Heller G, Marrinucci D, Fleisher M, Dittamore R (2016) Association of AR-V7 on circulating tumor cells as a treatment-specific biomarker with outcomes and survival in castration-resistant prostate cancer. JAMA Oncol 2:1441–1449
105. Shaffer PL, Jivan A, Dollins DE, Claessens F, Gewirth DT (2004) Structural basis of androgen receptor binding to selective androgen response elements. Proc Natl Acad Sci U S A 101:4758–4763
106. Simons SS (2010) Glucocorticoid receptor cofactors as therapeutic targets. Curr Opin Pharmacol 10:613–619
107. Tan MH, Li J, Xu HE, Melcher K, Yong EL (2015) Androgen receptor: structure, role in prostate cancer and drug discovery. Acta Pharmacol Sin 36:3–23
108. Tepper CG, Boucher DL, Ryan PE, Ma AH, Xia L, Lee LF, Pretlow TG, Kung HJ (2002) Characterization of a novel androgen receptor mutation in a relapsed CWR22 prostate cancer xenograft and cell line. Cancer Res 62:6606–6614
109. Tien AH, Sadar MD (2018) Order within a disordered structure. Structure 26:4–6
110. Tien AH, Sadar MD (2021) Cyclin-dependent kinase 4/6 inhibitor palbociclib in combination with ralaniten analogues for the treatment of androgen receptor-positive prostate and breast cancers. Mol Cancer Ther. molcanther.0411.2021
111. Ueda T, Mawji NR, Bruchovsky N, Sadar MD (2002) Ligand-independent activation of the androgen receptor by interleukin-6 and the role of steroid receptor coactivator-1 in prostate cancer cells. J Biol Chem 277:38087–38094
112. Umesono K, Evans RM (1989) Determinants of target gene specificity for steroid/thyroid hormone receptors. Cell 57:1139–1146
113. Wang L, Wu Y, Zhang W, Kannan K (2012) Widespread occurrence and distribution of bisphenol A diglycidyl ether (BADGE) and its derivatives in human urine from the United States and China. Environ Sci Technol 46:12968–12976
114. Wang Y, Lonard DM, Yu Y, Chow DC, Palzkill TG, O'Malley BW (2011) Small molecule inhibition of the steroid receptor coactivators, SRC-3 and SRC-1. Mol Endocrinol 25:2041–2053
115. Ward JJ, Sodhi JS, Mcguffin LJ, Buxton BF, Jones DT (2004) Prediction and functional analysis of native disorder in proteins from the three kingdoms of life. J Mol Biol 337:635–645
116. Wardell SE, Kwok SC, Sherman L, Hodges RS, Edwards DP (2005) Regulation of the amino-terminal transcription activation domain of progesterone receptor by a cofactor-induced protein folding mechanism. Mol Cell Biol 25:8792–8808
117. Wright PE, Dyson HJ (2015) Intrinsically disordered proteins in cellular signalling and regulation. Nat Rev Mol Cell Biol 16:18–29
118. Xie H, Vucetic S, Iakoucheva LM, Oldfield CJ, Dunker AK, Uversky VN, Obradovic Z (2007) Functional anthology of intrinsic disorder. 1. Biological pro-

cesses and functions of proteins with long disordered regions. J Proteome Res 6:1882–1898
119. Yamamoto KR (1985) Steroid receptor regulated transcription of specific genes and gene networks. Annu Rev Genet 19:209–252
120. Yang J, Young MJ (2009) The mineralocorticoid receptor and its coregulators. J Mol Endocrinol 43:53–64
121. Yang YC, Banuelos CA, Mawji NR, Wang J, Kato M, Haile S, Mcewan IJ, Plymate S, Sadar MD (2016) Targeting androgen receptor activation function-1 with EPI to overcome resistance mechanisms in castration-resistant prostate cancer. Clin Cancer Res 22:4466–4477
122. York B, O'Malley BW (2010) Steroid receptor coactivator (SRC) family: masters of systems biology. J Biol Chem 285:38743–38750
123. Yu X, Yi P, Hamilton RA, Shen H, Chen M, Foulds CE, Mancini MA, Ludtke SJ, Wang Z, O'Malley BW (2020) Structural insights of transcriptionally active, full-length androgen receptor coactivator complexes. Mol Cell 79(812-823):e4
124. Zhou J, Zhao S, Dunker AK (2018) Intrinsically disordered proteins link alternative splicing and post-translational modifications to complex cell signaling and regulation. J Mol Biol 430:2342–2359