Review

Small Molecule Inhibitors Targeting the "Achilles' Heel" of Androgen Receptor Activity

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Abstract

Androgen ablation therapy remains the gold standard for the treatment of advanced prostate cancer, but unfortunately, it is not curative, and eventually the disease will return as lethal castration-resistant prostate cancer (CRPC). Mounting evidence supports the concept that development of CRPC is causally related to continued transactivation of androgen receptor (AR). All current therapies that target the AR are dependent on the presence of its C-terminal ligand-binding domain (LBD). However, it is the N-terminal domain (NTD) of the AR that is the 'Achilles' heel' of AR activity, with AF-1 being essential for AR activity regardless of androgen. Recent efforts to develop drugs to the AR NTD have yielded EPI-001, a small molecule, sintokamide peptides, and decoys to the AR NTD with EPI-001, the best characterized and most promising for clinical development based upon specificity, low toxicity, and cytoreductive antitumor activity.

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Background

Androgens such as testosterone and dihydrotestosterone mediate their biological effects through the androgen receptor (AR). In adult males, the testes produce the majority of androgen with some contribution from the adrenal glands. Androgens play a role in a wide range of developmental and physiologic responses and are involved in male sexual differentiation, maintenance of spermatogenesis, and male gonadotropin regulation. The growth and survival of the prostate is dependent on androgen. When androgens increase in males during puberty, there is an increase in growth of the prostate gland, and in adult males when androgens are reduced by castration, there is involution of the prostate and apoptosis of prostate epithelial cells. Thus, the prostate gland is an androgen-dependent organ in which androgens are the predominant mitogenic stimulus. This dependency of the prostate epithelium on androgens provides the underlying rationale for treating advanced prostate cancer with androgen ablation.

Castration-Resistant Prostate Cancer

Prostate cancer is the most frequently diagnosed noncutaneous tumor in Western men. Primary therapies such as radical prostatectomy and radiation for localized, low grade tumors generally result in low mortality rates. Unfortunately, high grade tumors of Gleason score 7 and above have elevated recurrence rates, even when it seems that the tumor has been successfully contained with primary therapy. Approximately 20 to 40% of prostate cancer patients treated with radical prostatectomy will experience tumor recurrence. Once the tumor has recurred, usually manifest by an increase in serum prostate-specific antigen (PSA), androgen ablation therapy is provided to most patients. Androgen ablation therapy is achieved through either orchiectomy (surgical castration) or application of gonadotropin-releasing hormone analogues (chemical castration), which both cause a transient reduction in tumor burden concomitant with a decrease in serum PSA. Unfortunately, the malignancy will eventually begin to grow again in the absence of testicular androgens to form castration-resistant disease [castration-resistant prostate cancer (CRPC)]. CRPC is biochemically characterized before the onset of symptoms by a rising titer of serum PSA. Most patients succumb to CRPC within 2 to 3 years of biochemical failure. All current therapies directed at AR target its C-terminal ligand-binding domain (LBD) and eventually fail. Treatments not targeting AR [e.g., docetaxel or sipuleucel-T (Provenge, Dendreon)] result in an increased life expectancy of 2 to 4 months.

Androgen Receptor in Castration-Resistant Prostate Cancer

Mounting evidence supports the concept that CRPC remains dependent upon AR signaling. Many of the same genes that are increased by androgens in androgen-dependent prostate cancer xenografts become elevated in CRPC, such as PSA. AR protein translocates from the cytoplasm to the nucleus when activated by androgen or alternative signaling pathways (1). Thus, the detection of nuclear localization of AR in CRPC supports that the AR may continue to be transcriptionally active in the absence of testicular androgens. Additional support for the AR continuing to play a role in CRPC includes the following: amplification of the AR gene and/or
increased expression of AR (2–4); delayed onset of CRPC by altering the timing and sequence of the use of antiandrogens and additional responses with CYP17 inhibitors that block the synthesis of androgen (5, 6); and the fact that AR expression is essential for proliferation and tumor growth (4, 7). Finally, it is known that AR can be activated through its N-terminal domain (NTD) in the absence of androgen by stimulation of the cAMP-dependent protein kinase (PKA) pathway, interleukin-6 (IL-6), and by bone-derived factors (1, 8, 9).

**Androgen receptor as a therapeutic target**

The AR has distinct functional domains that include the C-terminal LBD, a DNA-binding domain (DBD), and an NTD. The AR DBD has been crystallized, which would allow for rational drug design, but the high degree of homology of this domain with other steroid hormone receptors may predict poor specificity and toxic side effects. The transcriptional activity of most steroid hormone receptors is predominantly through the activation function (AF)-2 region in the LBD, the exception being the AR in which it is the AF-1 region in the NTD that contributes most of the transcriptional activity (10–12). AR LBD functions independently of the NTD and can still bind ligand even if the AF-1 region is deleted or mutated; however, no transcriptional activity can be achieved without the AF-1 region in the NTD.

Currently, all conventional therapy has concentrated on androgen-dependent activation of the AR through its C-terminal LBD. These therapies include reduction of androgen that binds to the LBD by chemical or surgical castration and application of antiandrogens (Fig. 1A). Unfortunately, castration does not completely eliminate levels of androgen in metastatic prostate cancer tissue (13). These residual levels of androgen have raised interest in the roles of adrenal androgens, transport of androgens, and/or de novo synthesis of androgen from cholesterol precursors by prostate cancer cells. Thus, there is renewed interest in inhibitors of androgen synthesis. These inhibitors include ketoconazole and, currently in clinical trials, the 17,20-lyase/CYP17 inhibitor abiraterone, TOK-001, and TAK-700. In addition to inhibiting 17,20-lyase activity to reduce synthesis of androgen, ketoconazole and TOK-001 (VN/124–1) also have antiandrogen activity. Antiandrogens competitively bind AR LBD. Antiandrogens used for the clinical management of prostate cancer include bicalutamide, flutamide, nilutamide, cyproterone acetate, and investigational compounds MDV3100 and ARN-509. Although castration and antiandrogens are effective initially, it is unclear why these approaches eventually fail. Suspected mechanisms include amplification or overexpression of AR; gain-of-function mutations allowing AR to be activated by steroids or antiandrogens, although most of these mutations are considered rare events; ligand-independent activation by growth factors, cytokines, or kinases; overexpression of AR coactivators; intracellular signaling by increased intratumoral androgens; and/or expression of constitutively active splice variants of AR that lack LBD that may be expressed solely or in mixed populations with full-length receptor to form a heterodimer (Fig. 1B). Most studies continue to develop antagonists to AR LBD, and specifically, these include (a) the allosteric pocket and AF-2 activity (14); (b) *in silico* drug repurposing procedure for identification of nonsteroidal antagonists (15); and (c) coactivator or corepressor interactions (16–19).

**Androgen receptor N-terminal domain as a target for drug development**

AR NTD contains several repeat regions: polyglutamine between residues 58 and 89; polyproline between residues 371 and 381; and polyglycine between residues 449 and 472. The transcriptional activity of the AR in response to ligand requires the NTD AF-1 (Tau1), which is between residues 101 and 370 with core sequence LKDL182 and does not play a significant role in ligand-independent activation of the AR (8). Tau5 is between residues 360 and 485 with core sequence WHTL149 and is considered to be important for activity in the absence of androgen (20). The AR NTD is flexible with a high degree of intrinsic disorder making it impossible to be used for structure-based drug design. The AR AF-1 region has characteristics of collapsed disorder, meaning that it is predicted to have some proportion of secondary structure, but not a stable tertiary structure. Induced folding of the AR NTD is thought to require interactions with other proteins including bridging factors and the basal transcriptional machinery to result in active transcription. A recent development is the acceptance of intrinsically disordered proteins and regions as attractive drug targets to prevent protein–protein interactions. An example is the nutlins that target the intrinsically disordered region of p53. When Mdm2 binds to the intrinsically disordered region (amino acid residues 13 to 19) of p53, a helical structure is formed that binds into the deep groove on the surface of Mdm2. As discussed by Uversky and colleagues, interaction between Mdm2 and p53 involves a disorder-to-order transition upon binding that would thermodynamically favor blocking by small molecule competitors (21). Other examples of therapeutics targeting protein–protein interactions include GX015–070 (Obatoclax, Gemin X), which interacts with the hydrophobic groove of bcl2 proteins to prevent binding of BH3 peptide domain of proapoptotic BCL2 members; inhibitors to XIAP-Diablo interactions and Grb2 SH2 domains; sulindac to prevent RAS-RAF interactions; and inhibitors of WNT pathway targets frizzled-dishevelled (Fj9 inhibitor) and β-catenin–CREB-binding protein (CBP; ICG-001).

For most of these interactions, crystal structures of at least one of the proteins have aided in the development of drug leads by permitting docking and virtual screening. Because AR NTD has not been crystallized and is intrinsically disordered, drug development is labor intensive and requires assays that test each drug empirically. However, despite these difficulties, 3 separate classes of inhibitors of the AR NTD have recently been described: EPI-001, a small molecule (22); sintokamides, which are peptides isolated from the marine sponge *Dysidea* species (23); and a decoy peptide to the AR NTD (24).

**Mechanism of action of small molecule inhibitors of androgen receptor N-terminal domain**

Deletion experiments have shown that the NTD is essential for transcriptional activity of the AR in response to ligand as
well as in the absence of ligand (10). Thus, the AR NTD is the "Achilles’ heel" of the AR transcriptional activity. Indeed, EPI-001 inhibited AR activity induced by androgen (Tau1), and in the absence of androgen (Tau5) under serum-free conditions (no cholesterol precursors that are required for de novo synthesis of androgens), by forskolin, which stimulates PKA activity, IL-6, and by factors secreted by osteoblasts (bone-derived factors; ref. 22). EPI-001 also inhibited constitutively active AR devoid of a LBD (22), which implies that the mechanism of action of EPI-001 involves a critical aspect of AR transcriptional activity and is not dependent on the presence of the LBD. Consistent with EPI-001 not binding
Inhibitors to the androgen receptor

The NTD has been shown to be a viable target for in vivo intervention as first indicated by application of decoy molecules encoding residues 1 to 558 of the AR NTD (AR1–558 ref. 24), and then recently using EPI-001 (22). Decoy AR1–558 inhibits full-length AR and blocks both androgen-dependent and CRPC tumor growth, most likely by a mechanism of mopping up essential proteins required for transcriptional activity (24). Development of shorter decoy peptides (~100 amino acids in length) to the AR NTD that retain specificity for AR and still have antitumor activity has been difficult due to multiple factors, including peptide lability and the possible requirement of multiple, nonlinear regions of the AR NTD necessary for protein–protein interactions. Systemic delivery of AR NTD decoys to target intracellular full-length AR in a clinical setting also could be a significant challenge. Small molecule inhibitors such as EPI-001, or potentially sintokamides, seem to overcome many of these hurdles for therapeudic development for AR NTD decoys.

Consistent with inhibiting AR activity, EPI-001 blocks AR-dependent proliferation in human prostate cancer cells that express AR and has no effect on the proliferation of cells that do not express functional AR or do not rely on the AR for growth and survival (22). Consistent with EPI-001 blocking the androgen-AR axis, intravenous injection of EPI-001 significantly reduced the weight of benign prostates from noncastrated mature mice compared with control-treated animals (22). EPI-001 also blocked the growth of prostate cancer xenografts in the presence of androgen (noncastrated mature male mice) and, most importantly, caused tumor regression of CRPC. Male mice bearing LNCaP xenografts treated with EPI-001 by i.v. injection had tumors that were less than half the size of tumors in controls after only 2 weeks of treatment (22). Consistent with EPI-001 having specificity for AR, EPI-001 had no effect on PC3 human prostate cancer xenografts that are insensitive to androgen and do not express functional AR (22). These data support that EPI-001 is specific to the AR and does not affect cells that do not depend on functional AR for growth and survival. No toxicity was observed in animals treated systemically with EPI-001 as determined by no loss of body weight, no changes in behavior, and no pathologic changes in the histology of internal organs (22). Pharmacokinetic studies show that EPI-001 has 86% oral bioavailability. Therefore, longer treatment periods can be obtained by oral dosing rather than i.v. and should provide an indication if tumors that express AR will completely regress in response to EPI-001.

EPI-001 inhibits constitutively active androgen receptor lacking the ligand-binding domain

A potential mechanism for resistance to antiandrogens and castration may involve expression of constitutively active splice variants of AR that lack LBD. Increased expression of constitutively active AR splice variants that are devoid of LBD has recently been described and is associated with earlier disease recurrence and death (25, 26). Expression of constitutively active AR occurs with high frequency in CRPC, and expression of these variants increases in response to reduced tissue levels of androgen (27). This finding suggests that the androgen environment plays a critical role in the expression of these variants. Further reduction of prostate tissue levels of androgen by CYP17 inhibitors may result in elevated levels of expression of constitutively active variant leading to CRPC and treatment failure. To date, two different variants have been detected in clinical tissue, ARv567es and ARV7/AR3 (25–27). Expression of ARv567es was most commonly detected in approximately 43% of 46 metastases that expressed AR that were obtained from patients who succumbed to CRPC (27). Transfection of ARv567es cDNA yields tumors that are resistant to castration; however, the ratio of expression of full-length AR to variant seems to be an important indicator of tumor response to castration (27). MDV3100, an antiandrogen in clinical trials, which binds to
the AR LBD, was tested in LNCaP xenografts transfected with AR-V7, such that these tumors should represent a mixed population of full-length AR and variant (28). Unfortunately, it was unclear whether there was, indeed, maintained expression of the variant in vivo because none of the growth advantage in response to castration was observed as reported by others with expression of the variant. Western blot analysis using an antibody to the NTD cannot distinguish degraded full-length AR from variant in the harvested tumors, and unfortunately, non-transfected tumors were not analyzed. Difficulty forcing expression of significant levels of AR-V7 protein seems to be a challenge, thereby complicating interpretation of the in vivo MDV3100 data in transfected LNCaP cells. Xenograft models using human tumors that naturally express predominantly ARv567es, such as LuCaP 86.2 (27), should provide a more physiologically relevant approach for determining the effects of MDV3100 and other AR antagonists on CRPC expressing AR variant lacking the LBD. Because EPI-001 inhibits the AR NTD and prevents essential N/C interaction of AR, it should directly block the activity of these variants, as well as prevent interaction of the variant with the full-length AR. Indeed, EPI-001 was effective at blocking the activity of a constitutively active deletion mutant, ARI-653, which contains the NTD, DBD, and hinge region, but not the LBD (22). It will be of interest to determine if EPI-001 also inhibits naturally occurring splice variants and mixtures of variant and full-length AR, as predicted from its mechanism of action. If so, EPI-001 may be the first inhibitor available for tumors that solely express variants, which occurs in 20% of metastases (27). Combination therapies using an anti-androgen, such as MDV3100 combined with EPI-001, may yield synergistic or additive responses in patients who generally have multiple tumors with varying ratios of full-length AR and variants.

Future Directions

Although the discovery of androgen ablation therapy for the treatment of prostate cancer was more than 50 years ago, there is still continued drug development targeted at the AR and androgen axis. Resurgence of interest in developing selective CYP17 inhibitors and more potent antiandrogens is the result of recent impressive clinical data being obtained from abiraterone and MDV3100. However, these inhibitors that ultimately interfere with AR LBD by either reducing ligand or direct interaction also seem to eventually fail, and AR transactivation is resumed. The discovery of significant expression of constitutively active splice variants of the AR lacking the LBD in CRPC tissue emphasizes the urgency to develop inhibitors of the AR NTD. EPI-001, sintokamides, and decoy AR1 each target the AR NTD and have each shown significant inhibition of AR with antitumor activity. Developing inhibitors to the intrinsically disordered NTD provides a novel concept in the field of steroid hormone receptor therapy, which has previously concentrated on targeting the C-terminus LBD.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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