Tip110, the Human Immunodeficiency Virus Type 1 (HIV-1) Tat-interacting Protein of 110 kDa as a Negative Regulator of Androgen Receptor (AR) Transcriptional Activation

Ying Liu, Byung Oh Kim, Chinghai Kao, Chaeyong Jung, James T. Dalton, and Johnny J. He

From the Department of Microbiology and Immunology, the Walther Oncology Center, the Department of Urology, and the Department of Medicine, Indiana University School of Medicine, Indianapolis, Indiana 46202, and the Division of Pharmaceutics, College of Pharmacy, Ohio State University, Columbus, Ohio 43210, and the Walther Cancer Institute, Indianapolis, Indiana 46206

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Upon binding to androgen, androgen receptor (AR) can activate expression of target genes through its direct binding to the androgen-responsive elements (AREs), which are located within the target gene promoters and/or enhancers. A number of cellular proteins have been identified as co-regulators to regulate this transactivation process. One common structural feature among these co-regulators is the presence of the LXXLL motif (X, any amino acid), the so-called nuclear receptor (NR) box, through which binding of these regulatory proteins to AR occurs. We have recently shown that Tip110 functions to potentiate the transactivation activity of human immunodeficiency virus type I (HIV-1) Tat protein. In this study, we report that Tip110 is a potent AR-binding protein that can suppress AR activity. Tip110 bound to AR in an NR box-dependent manner and inhibited AREs-mediated reporter gene expression. The inhibitory effects were abolished by removal of the NR box. Moreover, knock-down of the constitutive Tip110 expression significantly augmented AR transcriptional activation. In agreement with these findings, Tip110 overexpression blocked the prostate-specific antigen (PSA) gene, a well characterized target gene of AR from expression in LNCaP cells. Further analysis revealed that Tip110 prevented the complex formation between AR and AREs. Taken together, these results indicate that Tip110 is a negative regulator of AR transcriptional activation, and may be directly involved in AR-related developmental, physiological, and pathological processes.

The steroid hormone androgen exerts its biological functions through the androgen receptor (AR) (1, 2). Androgen-bound AR serves as a transcription factor and mediates gene expression in a number of cellular processes. These include male sexual differentiation, maturation, and spermatogenesis, and primary prostate cancer growth in prostate cancer patients (3–6). Thus, it is conceivable that changes in AR, androgen, or interaction between AR and androgen have been noted in male infertility, androgen insensitivity, and tumorigenesis.

Structurally, AR is divided into four functional domains: the NH2-terminal transactivation domain (or A/B domain) the DNA binding domain (DBD), hinge region, and the COOH-terminal ligand binding domain (LBD) (7). Two transcriptional activation functions have been identified in AR. One is at the NH2-terminal (AF1) and functions in a ligand-independent manner, whereas the other located within the LBD requires AR ligand for its function (AF2) (8–10). The DBD consists of two zinc fingers that recognize specific DNA consensus sequences called AR-responsive elements (AREs) (11). AR binds to AREs as a homodimer, or in a heterodimer with other steroid hormone receptors. Androgen functions by binding to and inducing a conformational change in AR. This is believed to facilitate interaction between the NH2 and COOH terminus of AR and recruitment of AR co-regulators and subsequent regulation of target genes (12, 13).

A number of co-regulatory proteins have been identified to interact with AR and modulate AR transcriptional activation in a positive manner (co-activators) or a negative manner (co-repressors) (14). In general, the co-regulators themselves do not possess specific DNA binding property (12, 15, 16). There are multiple mechanisms by which co-regulators affect AR transcriptional activation. Some co-regulators function with AR at the target gene promoter to facilitate DNA occupancy, chromatin remodeling, or recruitment of general transcription factors associated with the RNA polymerase II holoenzyme. Some co-regulators exert their effects through direct regulation of AR folding, stability, and nuclear translocation, ligand binding, intramolecular interaction between the NH2 and the COOH domains, or signal transduction (14). In addition, several co-regulators have also been found to directly modulate the ability of AR to bind its recognition sequence AREs and subsequently to transactivate gene expression (17, 18).

A number of co-regulators of steroid hormone receptors including AR have been found to contain a short a-helical sequence, the LXXLL motif (where X is any amino acid), or the so-called nuclear receptor (NR) box (19–28). The common NR box is often necessary and sufficient to mediate the interactions...
between co-regulators and the nuclear receptor LBD (29, 30). X-ray crystallographic studies of the highly conserved LBD of NRs have revealed that ligand binding induces movement of helix 12 in the LBD against helices 3 and 5 and subsequently creates a small hydrophobic cleft to be recognized and bound by the coregulators (31–33). In the case of AR, this motif has also been found to mediate ligand-dependent interactions between the LBD and the other AR co-regulators (34–37). We have recently identified a novel human immunodeficiency virus type 1 Tat-interacting protein Tip110. Sequence analysis indicates an NR box present at amino acid residues (aa) 118–122 of Tip110. In this study, we have shown that the NR box-containing Tip110 protein bound to AR and negatively regulated AR transcriptional activation activity. These studies may have important implications for understanding regulation of AR functions in normal physiological processes, as well as for developing therapies for AR-related pathophysiological disorders including cancers.

**MATERIALS AND METHODS**

**Cell Lines and Cell Transfection**—293T cells and LNCaP cells were purchased from American Tissue Culture Collection (ATCC, Manassas, VA) and were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (HyClone, Logan, UT) at 37 °C with 5% CO2. For luciferase reporter gene assay and detection of prostate-specific antigen (PSA) expression, LNCaP cells were maintained in DMEM supplemented with 10% dextran charcoal-stripped fetal bovine serum at 37 °C with 5% CO2. 293T cells were transfected by the standard calcium phosphate precipitation method, as previously described (38). LNCaP cells were transfected using the LipofectAMINE 2000 according to the manufacturer's instructions (Invitrogen). Transfection medium was replaced with fresh medium containing 100 μM R1881 (Sigma) 10–12 h after transfection unless otherwise stated, and cells were harvested for protein analysis by Western blot or immunoprecipitation followed by Western blot, or for assay of the luciferase reporter gene activity, or for isolation of total RNAs and RNA analysis by reverse transcription-PCR (RT-PCR) or Northern blot. In all transfections, pcDNA3 was used to equalize the amount of DNA transfected, and pTKgal was included to normalize the variations in transfection efficiency.

**Plasmids**—Plasmid pTip110.His, the deletion mutants ΔCT, ΔNLS, ΔRMR, and ΔNLSΔRMR, pAsp-Tip110, and pTKgal are described elsewhere (38). Tip110 mutants; L118A (wild-type), L581A, L582A, L583A, L583L, L583V, L583G, L583D, L583E, L583Q, L587A (non-conservative substitutions), L581V, L581A, L582V, L582A, L583V, L583A, L581Q, L581V, L582Q, L582V, L583Q, L583V, L587A (conservative substitutions), and L581Q, L582Q, L583Q, L587Q (non-conservative substitutions) were prepared by directed mutagenesis and verified by DNA sequencing. Then, the mutant Tip110 constructs were inserted into the mammalian expression vector pBlueBacHis2A-hAR expressing the full-length human AR is described previously (40). Preparation of Whole Cell Lysates, Immunoprecipitation, and Western Blot—Cells were lysed in a buffer containing 10 mM HEPES, 150 mM NaCl, 1% Triton X-100, 0.1% SDS, 0.2% sodium azide, 0.004% sodium deoxycholate, 1 mM sodium orthovanadate, and incubated on ice for 10 min. Whole cell lysates were obtained by centrifugation and removal of the cell debris. For Western blots, cell lysates (25 μg of protein) or immunoprecipitates (500 μg of protein) were electrophoretically separated on 10% SDS-PAGE and analyzed by immunoblotting using mouse anti-AR antibodies (Santa Cruz Biotechnology, Santa Cruz, CA), anti-AR antibodies (Santa Cruz Biotechnology), or anti-β-actin antibody (Sigma) at dilutions recommended by the manufacturers, followed by appropriate horseradish peroxidase-conjugated secondary antibodies, and then visualized with the ECL system (Amersham Biosciences).

**RNA Analysis by RT-PCR and Northern Blot**—Total RNA was extracted from LNCaP cells using the TRIzol reagent (Invitrogen) according to the manufacturer's instructions. 0.2 μg of total RNA was reverse-transcribed and PCR-amplified with PSA-specific 5′-GCCAGGTGCTT-GTACCGCTTCC-3′ and 5′-CACCAGGGACCGGTCTTCTTG-3′ using the Titan One Tube RT-PCR system (Roche Diagnostics, Indianapolis, IN). The PCR program consisted of 2 min of denaturation at 94 °C, 10 cycles of amplifications with 45 s of elongation time followed by 25 cycles of amplifications with 45 s of elongation time plus 5 s for each cycle. The expected PCR DNA products were about 522 bp in length. The human β-actin gene was included in the RT-PCR as an internal control, with primers 5′-AGCCACCTGTGTTGGCGTACAGGTC-3′ and 5′-CGGTCATCACCATTGGCCAATCAG-3′, and the expected PCR DNA was 139 bp in length. For Northern blots, total RNA was extracted from LNCaP cells as described above, and 25 μg of RNA was fractionated on a 1.2% denaturing agarose gel. The gel was stained with ethidium bromide for 18 and 28 S rRNAs, and the PSA mRNA was detected by hybridization with the 32P-labeled 522 bp PSA probe (the RT-PCR product). The probe was prepared using the random-primed DNA labeling kit (Roche Diagnostics).

**Recombinant Protein Purification**—GST-Tip110 and GST proteins were expressed in Escherichia coli BL21 strain. Bacteria harboring the pGEX-Tip110 or pGEX-4T-3 plasmid were grown in LB medium containing 50 μg/ml ampicillin at 30 °C overnight, 1 mM isopropyl-1-thio-β-galactoside was added to the culture at 1 h after cell growth 

**RESULTS**

Tip110 Binding to AR—We have recently identified a new protein Tip110 as a co-transactivator of human immunodeficiency virus type 1 (HIV-1) Tat protein (38). Further examination of the Tip110 primary amino acid sequence identified an LXXLL motif between amino acid positions 118 and 122. Various androgen receptor co-regulators contain this LXXLL motif, which is recognized by and recruited through the activation...
function 2 (AF2) domain of AR. To investigate whether Tip110 binds to AR, 293T cells were transfected with pAR.Flag (10 μg, lane 2), pTip110.His (10 μg, lane 3), or both (10 μg each, lane 4) and harvested 48 h after transfection for whole cell lysates. pcDNA3 (lane 1) was used in mock transfection and also added to equalize the total amount of DNA among transfections. Cell lysates (25 μg of total protein) or immunoprecipitates (1 mg of total protein) were separated electrophoretically on 10% SDS-PAGE and transferred onto the Hybond-P membrane. The membranes were probed with anti-Flag or anti-His antibody, followed by appropriate secondary conjugates, and then developed with the ECL system. *, reactive IgG bands; WB, Western blot; IP, immunoprecipitation.

**Fig. 1. Direct binding of Tip110 to AR.** 293T cells were transfected with pAR.Flag (10 μg, lane 2), pTip110.His (10 μg, lane 3), or both (10 μg each, lane 4) and harvested 48 h after transfection for whole cell lysates. pcDNA3 (lane 1) was used in mock transfection and also added to equalize the total amount of DNA among transfections. Cell lysates (25 μg of total protein) or immunoprecipitates (1 mg of total protein) were separated electrophoretically on 10% SDS-PAGE and transferred onto the Hybond-P membrane. The membranes were probed with anti-Flag or anti-His antibody, followed by appropriate secondary conjugates, and then developed with the ECL system.

**Tip110, a Repressor of AR**

To determine the specific binding of Tip110 to AR and the role of the NR box of Tip110 in its binding to AR, we constructed a series of Tip110 deletion mutants including the mutant deleted for the NR box (Fig. 2A). Similarly, we transfected 293T cells with the AR expression plasmid in combination with each of Tip110 mutants and determined its binding to each of the Tip110 mutants by immunoprecipitation and Western blot. All mutants except the Tip110ΔRRM mutant were expressed at the expected molecular sizes and at comparable levels (Fig. 2B, top panel). Like the wild-type Tip110, deletion of the nuclear localization signal (ΔNLS), the RNA recognition motifs (ΔRRM), or the COOH terminus domain (ΔCT, amino acids 670–963) retained the ability of Tip110 to bind to AR (Fig. 2B, bottom panel). In contrast, NR box deletion (ΔNR) abolished Tip110 binding to AR (Fig. 2B, lane 3). Interestingly, the mutant deleted for both NLS and RRM (ΔNLSΔRRM) rendered Tip110 unable to bind to AR (Fig. 2B, lane 7), suggesting that in addition to the NR box, intramolecular interaction or appropriate conformation of Tip110 may also be required for Tip110 interaction with AR.

To ascertain whether the NR box is directly involved in Tip110 binding to AR, we sequentially mutated each of the leucine amino residues at amino acid positions 118, 121, and 122 to alanine within the NR box. Similarly, we co-expressed these site-directed Tip110 mutants with AR and analyzed their interaction. The results showed no complex formation detected between AR and any of these three Tip110 mutants, although they were expressed at comparable levels (Fig. 2C, lanes 4–6). In addition, we also co-expressed the wild-type Tip110 with AR in 293T cells and cultured the transfected cells in medium containing dextran charcoal-stripped fetal bovine serum. The results showed no complex formation between Tip110 and AR in lysates prepared from these cells (Fig. 2C, lane 7). Taken together, these results demonstrated that the NR box was directly involved in Tip110 binding to AR and further support that Tip110 bound to AR in a specific manner.

**Inhibition of AR Transcriptional Activation by Tip110**—A number of AR co-regulators have been identified to interact with AR and function as either AR co-activators or co-repressors in AR-mediated gene expression. Our results showed complex formation between Tip110 and AR. Thus, we decided to investigate the effect of Tip110 on AR transcriptional activation. We took advantage of an AR reporter gene assay, which involves use of an AR-responsive DNA element-driven fruit fly luciferase reporter gene p4GRE.TATA-Luc containing 4 tandem repeats of AR-responsive DNA elements (39) and human prostate LNCaP cancer cells, constitutively expressing AR. LNCaP cells were transfected with p4GRE.TATA-Luc plasmid alone or in combination with Tip110 expression plasmid, and the transfected cells were allowed to grow in the absence or presence of a synthetic androgen R1881. As expected, addition of 100 nM R1881 increased AR-mediated Luc expression by about 3.5-fold (Fig. 3A). Expression of Tip110 resulted in a considerable reduction of AR-mediated Luc expression in the presence of R1881, and the inhibition appeared to be correlated with the amount of Tip110 DNA transfected (Fig. 3A). Nevertheless, Tip110 expression in the absence of R1881 showed little effect on the basal level of AR-mediated Luc expression (Fig. 3A). Moreover, the inhibitory effects of Tip110 were not caused by decreased levels of AR expression (Fig. 3B). Similar results were obtained in non-prostate cancer cells such as 293T cells by co-transfection of p4GRE.TATA-Luc, AR, and Tip110 (data not shown). These results suggest that Tip110 functioned as a repressor of ligand-dependent AR transcriptional activation.

**Ablation of Tip110 Inhibition of AR Transcriptional Activation by Removal of the NR Box**—Although the NR box has been shown to be important for AR binding to its co-regulators, its direct function on AR transcriptional activation remains largely inconsistent. For example, the NR box in Tip60 is directly involved in Tip60-induced AR transcriptional activation, but the NR box in p160 has no effect on AR transcriptional activation. Thus, we next determined whether NR-mediated Tip110 binding to AR is attributable to the inhibitory effect of Tip110 on AR transcriptional activation activity. LNCaP cells
were transfected with p4GRE.TATA-Luc in combination with each of the Tip110 mutants, and the AR-mediated Luc expression was determined in the presence of R1881. Tip110 mutants ΔCT, ΔNR, and ΔRRM exhibited Luc expression at a level similar to that of the wild-type Tip110, whereas both mutants ΔNR and ΔNLSΔRRM showed no inhibitory effect on Luc expression (Fig. 4). These results suggest that direct binding of Tip110 with AR is a prerequisite for Tip110-induced inhibition of AR transcriptional activation.

Enhancement of AR Transcriptional Activation by Tip110 Knock-down—Our previous studies have shown ubiquitous expression of Tip110 mRNA in a variety of human tissues and cell lines (41). To further characterize the potential roles of Tip110 in AR transcriptional activation, Tip110 expression was determined in LNCaP cells. Western blot analysis showed that Tip110 protein was expressed in LNCaP cells (Fig. 5A, lanes 1 and 2), which raised the possibility that Tip110 may be a constitutive repressor of AR. To test this possibility, we down-modulated constitutive Tip110 expression and determined whether it would result in increased AR transcriptional activation activity. We took advantage of the antisense RNA expression vector-based strategy, which has successfully and widely been used to study the function of a number of genes including Tip110 (41, 42). LNCaP cells were transfected with p4GRE.TATA-Luc and the anti-Tip110 antisense RNA expression plasmid pAs-Tip110 that expressed the Tip110 antisense cDNA in a reverse orientation in the context of pcDNA3 backbone, and the Luc expression was determined. Transient expression of the anti-Tip110 antisense RNA effectively down-modulated constitutive Tip110 expression in a dose-dependent manner, as assessed by Western blot using anti-Tip110 sera.
Corresponding with Tip110 down-modulation, a parallel increase of AR-mediated Luc expression up to about 5-fold was noted (Fig. 5B). These results further support the notion that Tip110 was a repressor of AR, and suggest an important role of Tip110 in regulating AR transcriptional activation. Higher amounts of pAs-Tip110 DNA resulted in a slight reduction of Luc expression, which was likely due to apparent nonspecific cytotoxicity.

Inhibition of PSA Gene Expression by Tip110

The best characterized androgen-responsive gene in LNCaP cells is the gene encoding PSA, which is expressed mainly in the human prostate and is up-regulated by androgen (43). PSA has been used as a prostate-specific tumor marker for monitoring prostate cancer (44) and as a model gene for studying the mechanisms by which AR-mediated transactivation occurs in prostate cells. Thus, we next examined the relationship between Tip110 expression and PSA expression in LNCaP cells. We first determined PSA mRNA levels. LNCaP cells were transfected with pTip110.His in the presence of R1881, and total RNA was isolated and determined for PSA mRNA. RT-PCR using PSA-specific primers showed that Tip110 expression resulted in a parallel reduction of PSA mRNA expression in LNCaP cells, but showed little effect on the levels of the housekeeping gene β-actin mRNA (Fig. 6A). Similar results were obtained by Northern blot using a 32P-labeled PSA-specific DNA probe (Fig. 6B). Subsequently, we determined whether the inhibitory effects on PSA mRNA levels were translated to protein expression. LNCaP cells were transfected with pTip110, and/or incubated in the absence or presence of R1881, and then examined for PSA protein expression by Western blot using an anti-β-actin antibody (A) or for luciferase activity assay (B). pcDNA3 was added to equalize the total amounts of DNA among transfections, and pTKβGal was included to normalize transfection variations among transfections. Data represent means ± S.E. of triplicate samples.

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human PSA antibody. Cells were also treated with forskolin as a positive control for PSA expression, because forskolin has been shown to be capable of up-regulating constitutive PSA protein expression. Addition of forskolin and R1881, as expected, induced PSA protein expression by about 5- and 3-fold, respectively (Fig. 6, lanes 2 and 3). In contrast, Tip110 protein expression completely reduced PSA protein to a basal level (Fig. 6C, lane 4). There were no apparent changes of β-actin protein (Fig. 6C, bottom panel). Taken together, these results demonstrated that Tip110 expression was able to inhibit AR-mediated target gene PSA expression at both the mRNA and protein levels.

**Blocking of AR Binding to AREs by Tip110**—Upon androgen binding and activation, the AR transactivates its target genes through direct binding to AREs located within the promoters of these genes including PSA (43). Thus, it was likely that Tip110-induced inhibition on AR transcription activity was caused by direct modulation of AR binding to AREs. We then performed the gel mobility shift assay to determine AR binding to AREs in the presence of Tip110. Co-incubation of full-length recombinant AR protein and complex 32P-labeled ARE DNA probe in the presence of R1881 led to complex formation between AR and ARE (Fig. 7, lane 2). The complex formation was not present in the absence of R1881 (Fig. 7, lane 1), although it was inhibited by the addition of unlabeled ARE DNA (Fig. 7, lanes 3–6), demonstrating the specificity of AR-ARE binding. Addition of purified recombinant GST-Tip110 fusion protein resulted in a dose-dependent inhibition in the formation of the complex (Fig. 7, lanes 8–10), whereas GST protein alone showed little effect (Fig. 7, lane 7). Moreover, no complex formation was detected between GST-Tip110 protein and ARE DNA (Fig. 7, lane 11). These results demonstrated that Tip110 impeded the complex formation between AR and its cognate DNA binding elements AREs, and suggest that this may account for Tip110-induced inhibition of AR transcriptional activation. In addition, requirement of R1881 for complex formation between AR and AREs suggests that conformational changes of AR following AR ligand binding may also be important for AR dimerization and/or subsequent AR binding to AREs within the target genes.

**DISCUSSION**

We originally identified Tip110 as an HIV-1 Tat-interacting protein of 110 kDa using yeast two-hybrid cloning. The presence of a single LXXLL motif or NR box within the primary amino acid sequence prompted us to investigate its potential function as a co-regulator of nuclear receptor AR. Many co-activators of nuclear receptors contain one or more NR boxes, through which their binding to nuclear receptors occurs. These include co-activators such as GRIP1, CBP, SRC-1, and Tip60 (14). However, whether the NR box plays an important role in regulation of AR transactivation differs among these co-activators. For example, the NR box located at the COOH terminus of Tip60 is required for up-regulation of Tip60-induced AR activation. Conversely, AR co-activators in the p160 family contain three NR boxes, but SRC1 in this family up-regulates AR...
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transactivation in an NR box-independent fashion. In this study, we showed that Tip110 directly bound to AR (Fig. 1) and that the binding was mediated by the NR box (Fig. 2). Interestingly, although removal of the NLS or RRM domain, which does not contain the NR box, did not affect Tip110 interaction with AR, the deletion mutant containing both NLS and RRM domains completely abolished binding. Nevertheless, removal of the entire COOH terminus did not show any effect. These results suggest that other intramolecular interactions of Tip110 may participate in its interaction with AR.

We then took advantage of the AREs-mediated reporter gene assay and found that overexpression of exogenous Tip110 resulted in inhibition of AREs-dependent reporter gene expression. However the wild-type or mutants that resulted in inhibition of AR-mediated reporter assay and found that overexpression of exogenous Tip110 with AR binding, deletion mutants lacking the NR box or both the NLS and RRM domains that lost AR binding affinity showed little inhibitory effect on AR-mediated reporter gene expression. Moreover the wild-type or mutants that retained AR binding exhibited comparable and strong inhibition (Fig. 4). Moreover, inhibition of AR transcriptional activation by the Tip110ΔNLS mutant (Fig. 4) indicates that Tip110ΔNLS binding to AR may have impeded AR nuclear localization and subsequent activation. We further used the antisense RNA knock-down technology to down-modulate constitutive Tip110 expression and found a significance increase in AREs-mediated reporter gene expression that apparently correlated with decreased expression of constitutive Tip110 (Fig. 5). Taken together, these results provide strong evidence to suggest that Tip110 is a potent negative regulator of AR transactivation.

PSA is expressed mainly in the human prostate and is the best characterized androgen-responsive gene in the prostate gland. It has been used as a model gene for studying the mechanisms by which AR-mediated transactivation occurs in prostate cells, as well as a useful marker for monitoring the prostate (43, 44). Three AREs have been identified in the PSA gene: ARE I and ARE II are in the ~630-bp promoter region, whereas ARE III resides in the enhancer region located ~4-kb upstream of the PSA transcription start site (45). Thus, to further determine the significance of Tip110 in AR transactivation, we determined the relationship between overexpression of Tip110 and PSA levels. As seen in the AREs-driven reporter gene assay, Tip110 overexpression led to a decrease in PSA mRNA levels as well as PSA protein levels (Fig. 6).

Although a number of AR co-regulators have been identified, a majority of them belong to AR co-activators and only a few AR co-repressors have been identified to date: cyclin D1 (46), calreticulin (17), HBO1 (14), TGIF (18), Smad3 (47), and Ebp1 (48). Calreticulin inhibits AR transactivation by interacting with AR DBD and as a result prevents AR binding to AREs. Two likely but not mutually exclusive mechanisms may exist. One is that Tip110 directly binds AR at the AR DBD and physically makes AR inaccessible to AREs. The other would be that Tip110 binds to AR at regions other than the AR DBD, which leads to formation of incorrect conformation within AR following androgen binding, subse-

quenty making it incapable of binding to AREs. Our results support the second possibility, since deletion of the DBD from AR did not abolish its ability to bind to Tip110 (data not shown).

Like other members of the nuclear receptor superfamily, the AR contains distinct structural and functional domains consisting of an NH2-terminal domain, a central DNA binding domain, and a COOH-terminal ligand binding domain. The NH2-terminal domain is the most variable between nuclear receptors in terms of both length and sequence (4, 50). The DBD of all members of the nuclear receptor superfamily consists of two zinc fingers that recognize specific DNA consensus sequences (14). Nonetheless, it has also been shown that the NR box displays varying degrees of nuclear receptor selectivity, which appear to be specified by the amino acid residues immediately flanking the NR box (30, 33, 51–54). Moreover, the biological significance of selective interactions between various nuclear receptors and co-regulators is largely unclear; however, selective interaction in vivo may play a role in tissue- and cell-specific effects (52). Therefore, whether Tip110 plays a similar role in other nuclear receptor transactivation functions remains to be investigated.

In summary, our results showed that Tip110 is a negative regulator of AR. Unlike these AR co-repressors that form a tripartite complex with AR and AREs, Tip110 aborted the complex formation between AR and AREs. Elucidation of Tip110 interaction with AR and its role as a negative regulator of AR transactivation function may likely shed light not only on understanding AR roles in normal physiological processes but also on intervening AR functions in pathophysiological processes such as prostate cancer. However, the true in vivo relevance of Tip110 as an AR-negative regulator should be established in intact animal models, such as Tip110 gene knock-out mice.

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