ROLE OF TRANSCRIPTIONAL ACTIVATION UNIT 5 (TAU5) IN MEDIATING TRANSCRIPTIONAL ACTIVITY OF ANDROGEN RECEPTOR SPLICE VARIANTS

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SARITA KUMARI MUTHA

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DEDICATION

This thesis is dedicated to my mentor and advisor Scott Dehm. This work would not have been possible without his support. I am very grateful for my time under his mentorship and the opportunity to learn from him.

ABSTRACT

The standard treatment for advanced prostate cancer is chemical castration, which inhibits the activity of the androgen receptor (AR). Eventually, prostate cancer reemerges with a castration-resistant phenotype (CRPC) but still depends on AR signaling. One mechanism of AR activity in CRPC is the synthesis of AR splice variants, which lack the ligand binding domain. These splice variants function as constitutively active transcription factors that promote expression of endogenous AR target genes and support androgen independent prostate cancer cell growth. Previous work has shown transcriptional activation unit 5 (TAU5) is necessary for ligand independent activity of the full length AR in low or no androgen conditions and that this activation is mediated by the WHTLF motif. The purpose of this study was to determine whether the TAU5 region was also important for regulating the constitutive activity of truncated AR variants. We generated deletion mutants of the AR variants and tested transcriptional activity by luciferase assay. We found that that the constitutive, ligand independent transcriptional activity of truncated AR variants was dependent on TAU5 for transcription. Surprisingly, we found that AR variants did not require WHTLF to mediate this ligand independent activity. We attempted to narrow down the region that may be important for variant transcriptional activity and found that deletion of amino acids 420-490 resulted in lower activity compared to AR 1/2/3 CE3. However, testing smaller regions of 420-490 did not elucidate a specific motif. These findings highlight TAU5 as a key AR domain through which AR activity could be inhibited for treatment of CRPC.

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LIST OF ABBREVIATIONS

AF-2: activation function-2 domain

AR: androgen receptor

ARA: androgen receptor associated

ARE: androgen response element

CGH: comparative genome hybridization

CRPC: castration-resistant prostate cancer

CTD: C-terminal domain

CTE: carboxyl-terminal extension

DBD: DNA binding domain

DHEAS: dehydroepiandrosterone

DHT: dihydrotestosterone

ER: estrogen receptor

ETS: E26 transformation-specific

HSP: heat shock protein

T: testosterone

TAU1: transcriptional activation unit 1

TAU5: transcriptional activation unit 5

LBD: ligand binding domain

LHRH: luteinizing hormone releasing hormone

N-C: N-terminal domain- C-terminal domain

NLS: nuclear localization signal

LIST OF ABBREVIATIONS

NTD: N-terminal domain

PIN: prostatic intraepithelial neoplasia

PSA: prostate-specific antigen

PTM: post-translation modification

SRC1: steroid receptor co-activator 1

SUMO: small ubiquitin-like modifier

INTRODUCTION

ANDROGEN RECEPTOR

Androgens are sex hormones responsible for the development and maturation of male reproductive organs and secondary sex characteristics [1]. They belong to the steroid hormone family and are synthesized from a 27 carbon cholesterol [2]. The primary synthesis of androgens occurs in the testis producing testosterone (T), which can be directly converted to dihydrotestosterone (DHT) [2]. A secondary area of synthesis is in the adrenal gland producing dehydroepiandrosterone sulfate (DHEAS), which is converted to T in peripheral tissue, and then converted to DHT. Testosterone, the major form of circulating androgens (90%), is converted to DHT by 5a-reductase in prostate and other reproductive tissue [1]. DHT binds to the ligand binding domain (LBD) of the androgen receptor (AR). Upon binding of DHT to the AR, the AR dimerizes, localizes to the nucleus, and binds DNA to induce and repress transcription of genes [3–5]. Prostate tissue depends on androgens for growth and survival. In other tissues, androgens induce differentiation and are essential for maintenance of male reproductive function, bone and muscle mass, hair growth, and behavior [5].

ANDROGEN RECEPTOR STRUCTURE

The AR is a 110 kDa nuclear receptor that belongs to the steroid receptor class I family [5], [6]. It is encoded by 8 exons and is 919 amino acids in length with variability in the poly glutamine and poly glycine stretch of the protein [5]. The AR gene is located on the X chromosome at Xq11-12 [7]. The domains of the AR are similar to other steroid receptors such estrogen receptor α (ERα), ERβ, and progesterone receptor in that they all contain the following: the amino-terminal domain (NTD), DNA

binding domain (DBD), the hinge region, and carboxyl-terminal domain (CTD) (Fig. 1) [5], [7].

The NTD is encoded by the entire 1st exon and is a fluid structure with no crystallographic or NMR based data currently available [5]. However, even in the face of difficult structural analysis, important domains have been carved out in the NTD. Jenster et al. demonstrated that transcriptional activation unit (TAU) TAU1 (amino acids 101-360) and TAU5 (amino acids 361-490) were two domains important for regulating transcriptional activity [8]. The NTD encompasses about 60% of the protein but has variable length due to the polyglutamine and polyglycine tracts [7].

Exons 2 and 3 encode the DBD. This domain is highly conserved among steroid receptors, it is approximately 80 amino acids long, and contains two zinc fingers which coordinate four cysteines. The first zinc finger is essential for the integrity, structure, and function of the DBD [9]. The second zinc finger contains a 5 amino acid motif known as the "D Box" which is important for DNA dependent dimerization [9].

Exon 4 encodes the hinge region. The hinge region is poorly conserved among the steroid receptors. Originally, the hinge region was described only as a flexible linker between the DBD and LBD [5]. It is now known to contain the nuclear localization signal (NLS) as well as the carboxyl-terminal extension (CTE) of the AR DBD [5]. These sequences in the hinge region allow for nuclear import as well as DNA selectivity, affinity, and transactivation potential of the AR [5].

Exons 5 through 8 encode the LBD. The structure of the ligand binding domain has been solved [10]. It contains a twelve-helical sandwich that forms a cavity for the ligand. When the AR is activated, helix 12 is repositioned causing the ligand binding

pocket to close and form a hydrophobic cleft [5]. This structure is also referred to as the activation fuction-2 (AF-2) domain. This region serves as a docking site for coactivators bearing a signature LxxLL motif [5].

CANONICAL MODEL FOR AR ACTIVATION

The unliganded AR localizes to the cytoplasm of the cell complexed with heat shock proteins (HSP) 90, 70, 56, and 23 [1]. Binding of DHT to the LBD results in a conformational change of the AR, allowing the AR to translocate into the nucleus [1]. Once in the nucleus, the AR dimerizes and binds to androgen response elements (AREs) found in the promoter and enhancer of genes [1], [11]. One well characterized gene is prostate-specific antigen (PSA), which has defined promoter and enhancer ARE elements [12]. Once situated at the ARE, the AR recruits coactivators such as p160 coactivators (SRC-1, GRIP1/TIF2, RAC3/ pCIP/ ACTR/ AIB1/ TRAM1), P/CAF, p300, or AR-associated (ARA) coactivators via the NTD and CTD to activate transcription [1]. The AR transcriptome includes genes important for the growth, proliferation, differentiation, and maintenance of prostate cells [13].

PROSTATE CANCER

Prostate cancer is the most frequently diagnosed cancer in men and second leading cause of male cancer death. In 2012 there will be an estimated 241,740 new cases and 28,170 deaths [14]. Prostate cancer progresses through several stages. Stage I and Stage II are found within the prostate. In Stage III, the cancer has spread out from the prostate to the seminal vesicles. In Stage IV the cancer has spread to nearby organs, tissues, or lymph nodes. While metastatic disease only accounts for <5% of diagnosed

patients, 40% of localized prostate cancer eventually recurs as metastatic disease despite local therapies such as surgery or radiation [15].

Utilizing histological methods, prostate cancer can also be described by the Gleason grading system. The precursor stage to prostate cancer is described as prostatic intraepithelial neoplasia (PIN) [16]. It is marked by cells with large nuclear and nucleolar structure, as well as an inversion of the basal and luminal compartments [16]. Progression into prostate cancer can be described by Gleason grades 2-10. This system focuses on five different histological patterns with decreasing differentiation of cells as the grade increases [17].

Currently, surgery and radiation are used to treat prostate cancer. Advanced stages of disease or metastatic disease are treated by surgical or chemical castration. There are several different drugs that are used for chemical castration. One group of drugs target testosterone production. Examples include luteinizing hormone releasing hormone (LHRH) agonists which target testosterone production by the testes, and more recently the CYP17A1 inhibitor abiraterone acetate which blocks androgen synthesis in the testes and adrenals [18]. AR antagonists, such as bicalutamide and more recently MDV3100 (enzalutamide) target the AR LBD [19]. These drugs bind to the LBD directly and alter interactions with AR cofactors, or alter translocation to the nucleus and DNA binding [15]. Castration generates many benefits including: shrinking tumor burden, decreasing prostate specific antigen (PSA) levels, enhancing quality of life, and improving overall survival [15]. However, treatment of advanced/metastatic disease by chemical castration eventually results in recurrence of disease within a median of 12-24 months [20].

AR DEPENDENT MECHANISMS FOR PROSTATE CANCER REEMERGENCE

The AR is a critical transcription factor in prostate cells that binds androgens and activates and represses genes essential for normal prostate cell differentiation, metabolism, and proliferation [13]. AR action is also critical in the development of prostate cancer. The initiating events of prostate cancer are AR driven events: the AR regulated gene TMPRSS2 fuses to the E26 transformation-specific (ETS) family of genes (ERG, ETV1, ETV4) [21–23]. This fusion results in aberrant AR driven expression of the ETS factors, which then induces PIN in animal models [23]. The dependence on the AR means that treatment requires surgical or chemical castration to target androgen production. When prostate cancer reemerges under castrate conditions it is referred to as castration-resistant prostate cancer (CRPC) to reflect the fact that AR signaling continues to be an important driver of tumor cell growth and survival. There are several mechanisms by which the prostate cancer becomes castration resistant.

1) *AR overexpression*

   Amplification of the AR gene can occur and has been detected by in situ hybridization and array based comparative genome hybridization (CGH) techniques. Gene amplification is associated with increased expression of AR protein, which can support hypersensitivity to low level of androgens [13], [24–26]. Of note, MDV3100 was specifically designed to treat disease marked by an overexpressed AR [27].

2) *AR gene mutations and altered ligand specificity*

   The AR can bind to many different agonists and antagonists [5], [13]. Various

AR point mutations have been reported in prostate cancer tissues, the majority of which are found in the LBD [25]. These mutations can alter ligand binding and specificity. For example, AR has been shown to bind glucocorticoids through a mutated androgen receptor (L701H and L701H&T877A) [28]. Another example is bicalutamide which acts as an antagonist but in castrate conditions can act inappropriately as an agonist [29]. The mutations W741C and W741L are associated with this bicalutamide antagonist-to-agonist change [30].

3) *AR Variants*

Another mechanism by which CRPC can arise is through the expression of truncated androgen receptor splice variants. AR variants lack the ligand binding domain but remain constituvely active ligand independent transcription factors [7]. These splice variants have been found to naturally occur in clinical samples of prostate cancer and prostate cancer cell lines [31]. They also have been shown to provide a growth advantage in CRPC-like conditions [7], [32].

AR VARIANTS

History

In 2002, Tepper et al. observed two AR protein species in the androgen independent prostate cancer cell line 22Rv1: full length AR (110 kDa) and a shorter isoform of the AR (75-80 kDa). Utilizing specific antibodies against portions of the AR (antibody mapping), it was determined that the 75-80 kDa species consisted of the NTD and DBD but lacked the LBD. In 2007, Libertini et al. found that an AR species similarly sized to the 75-80 kDa isoform was expressed in clinical prostate cancer [33]. In 2008, Dehm et al. demonstrated that in 22Rv1 cells, siRNA specifically targeting

exon 7 abolished expression of the full length (110 kDa) AR but the smaller AR isoform (75-80 kDa) remained unaffected, indicating an alternative splicing mechanism [34].

To date, 17 discrete truncated AR variant species have been described [32]. They were discovered by designing primers specific to exon 1 and then utilizing 3' RACE to discover novel 3' ends [31], [34–37]. This and similar approaches led to the discovery of two major types of AR variants: those arising from cryptic exon inclusion and those arising from exon skipping. Cryptic exon inclusion AR variants are generated by the splicing of cryptic exons located within introns 2 or 3 of the AR gene. (Fig 1) [7]. A single exon skipping variant has been discovered thus far and skips exons between 4 and 8 [7].

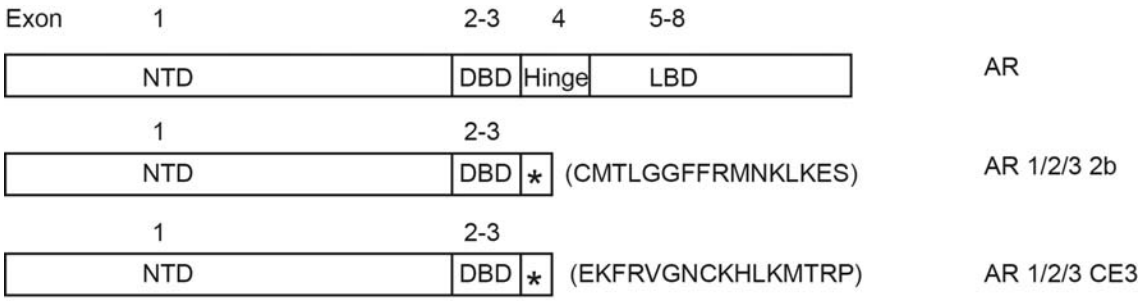


Figure 1: Example of two AR variants
AR 1/2/3 2b and AR 1/2/3 CE3 contain exons 1, 2, and 3, which encode the NTD and DBD but do not contain exons 4-8 which encode the hinge region and the LBD like full length AR. The * represents the unique c-terminal sequence for each variant and the sequence is indicated in parenthesis.

AR 1/2/3 2b

AR 1/2/3 2b was discovered by utilizing cDNA from 22Rv1 cells and performing 3' RACE. Cloning and sequencing yielded a novel 17bp sequence downstream of exon 2 now termed exon 2b [34]. In 22Rv1 cells exon 3 is duplicated and thus allows the splicing of AR 1/2/3 2b [34]. By performing luciferase assays in AR-null DU145 cells, Dehm et al. demonstrated that AR 1/2/3 2b was constitutively active in the absence of androgens [34]. Translation of AR 1/2/3 2b mRNA into protein was verified by knocking down the variant using a siRNA specific to 2b and then using a NTD specific antibody in 22Rv1 cells [34], as well a polyclonal antibody against the 2b derived sequence CMTLGGFFRMNKLKES [38].

AR 1/2/3 CE3

AR 1/2/3 CE3 is the best-characterized AR variant. Using RT-PCR assays, AR 1/2/3 CE3 was detected in clinical prostate cancer samples as well as normal prostate tissue [35]. AR 1/2/3 CE3 was expressed an average of 20 fold more in CRPC compared to hormone naïve prostate cancer [35]. AR 1/2/3 CE3 can also be specifically detected by an antibody that recognizes the unique CE3 derived sequence: EKFRVGNCKHLKMTRP [31]. Using this AR 1/2/3 CE3 specific antibody, it was determined that higher expression of AR 1/2/3 CE3 in hormone naïve prostate cancer predicted a higher chance of recurrence following surgical treatment [35]. Comparison of CRPC bone metastases samples to hormone naïve, primary and nonmalignant prostate tissue showed that there was an increase in mRNA encoding AR variants in the CRPC bone metastases samples [39]. Furthermore, increased AR variant expression was correlated with significantly poorer prognosis with only 2 months survival for high

expressing patients after palliative surgery for metastatic disease compared to 8 months for lower variant expressing patients [39].

In addition, AR 1/2/3 CE3 is found in prostate cancer cell lines. Utilizing the AR 1/2/3 CE3 specific antibody, AR 1/2/3 CE3 was shown to be expressed in VCaP, CWR22Rv1, and CWR-R1 cells [31], [35]. AR 1/2/3 CE3 was shown promote growth in low androgen conditions by overexpression in LNCaP cells, and AR 1/2/3 CE3 variant specific knockdown in 22Rv1 and CWR-R1 cells [31].

AR NTD: TRANSCRIPTIONAL ACTIVATION UNITS

Previous studies have shown that the LBD of the AR alone has relatively weak transcriptional activity and therefore it is the NTD that is responsible for transcriptional activity of the AR [40]. Indeed, truncated AR variants have been shown to have varying degrees of constitutive, ligand-independent transcriptional activity [31], [34], [41], [42].

In 1995, Jenster et al. generated a series of N-terminal deletions to determine regions of the NTD that were important for AR transcriptional activity [8]. Through this deletional analysis two regions were described: transcription activation unit (TAU)1 and TAU5 [8]. TAU1 is defined as amino acids 101-360 [8], [43]. Mutational analysis of TAU1 mapped the core sequence responsible for activity to the ^{178}LKDIL^{182} motif [44], [45].

TAU5 is defined as amino acids 361-490 in the NTD of the AR [8]. To study TAU5, in androgen dependent LNCaP cells, Dehm et al. transfected cells with an AR construct in which DNA binding domain was swapped for the GAL4 DNA biding domain [46]. This swap of DNA binding domains allowed the study of AR constructs and their transcriptional activity without interference from endogenous AR in luciferase

assays [46]. Deletion of TAU5 in the AR construct led to an increase in AR transcriptional activity in luciferase assays using a GAL4 specific reporter [46]. Alternatively, in the castration resistant C4-2 cell line, TAU5 deletion in AR constructs led to a loss of transcriptional activity in luciferase assays [46]. The same was seen in the androgen independent 22Rv1 cell line [46].

Through deletion analysis and site directed mutagenesis experiments, the Trp-His-Thr-Leu-Phe (WHTLF) motif within TAU5 that was identified and shown to be required for ligand independent AR activity [46]. Deletion or alanine point mutations of W, L, and F residues within this region resulted in loss of AR transcriptional activity [46]. This loss of transcriptional activity could be rescued by adding a 21 amino acid peptide containing WHTLF in place of TAU5 but not by adding a 21 amino acid peptide containing an AHTAA mutation [46]. Finally, stepwise addition of WHTLF motifs generated a stepwise increase in AR transcriptional activity in C4-2 cells [46]. One, two, or three copies of WHTLF-containing peptides tethered to the GAL4 DNA binding domain resulted in transcriptional activation of a GAL4-regulated luciferase reporter in both LNCaP and C4-2 cells lines [46]. These experiments demonstrated that WHTLF was a novel transcriptional activation motif [46].

PURPOSE OF STUDY

The purpose of this study was to determine whether the TAU5 domain, a region that is important for the ligand-independent activity in the full the length AR, was also important for regulating the constitutive activity of truncated AR variants AR 1/2/3 2b and AR 1/2/3 CE3. We hypothesize that TAU5 is important for the AR variant ligand independent activity and that this activity is mediated through the WHTLF motif.

MATERIALS AND METHODS

Cell Lines and Culture Conditions- DU145, LNCaP, Cos-7 cells were obtained from American Type Culture Collection (ATCC). DU145 cells are an AR-null human metastatic prostate cancer line. LNCaP are an AR-positive human prostate cancer cell line. Cos-7 cells are an AR-null African green monkey kidney fibroblast-like cell line. LNCaP cells were maintained in RMPI 1640 (Invitrogen) with 10% fetal bovine serum (FBS), 100 units/ml penicillin, and 100 ug/ml streptomycin in a 5% CO2 incubator at 37° C. The DU145 and Cos-7 cell lines were cultured in DMEM with 10% FBS. For androgen response experiments, cells were seeded in 10% charcoal stripped serum for 24 hours in RPMI 1640. Cells were then stimulated for 24 hours by replacement of growth medium with medium containing 1 nmol/L mibolerone (Biomol).

siRNA Reagents and Antibodies- Small interfering RNAs targeting exon 1were purchased from Dharmacon. The sense strand sequence for the siAR duplex is 5'-CAAGGGAGGUUACACCAAAUU -3'. Primary antibodies specific for AR NTD (N-20, catalogue #sc-816), ERK-2 (D-2, catalogue #sc-1647), and α-tubulin (catalogue #sc-23948) were purchased from Santa Cruz Biotechnology. An antibody specific for lamin A/C (4C11, catalogue #4777) was purchased from Cell Signaling.

Plasmids – Plasmid constructs harboring full length AR (p5HBhAR-A), ARΔTAU5, AR 1/2/3/2b, AR 1/2/3 CE3, MMTV LUC, -5746 PSA- LUC, and 4xARE-E4-LUC have been described [34], [38], [46], [47]. AR 1/2/3 CE3ΔTAU5 and AR1/2/3

2bΔTAU5 were generated by cutting the full length AR plasmid ARΔTAU5 [46] with Ecor1 and HindIII to liberate a NTD ΔTAU5 fragment. AR 1/2/3 CE3 [38] and 1/2/3 2b [34] were digested with Ecor1 and HindIII, dephosphorylated, purified and ligated with the ΔTAU5 fragment. AR 1/2/3/2b AHTAA and AR 1/2/3 CE3 AHTAA mutants were generated using AHTAA mut FWD and REV primers (Table 1) and the Site-Directed Mutagenesis Kit (Stratagene). Deletion constructs of AR 1/2/3 CE3 were generated by using the Site Directed Mutagenesis Kit (Stratagene). BssHII sites were generated by integrating the BssHII restriction recognition site into the primers pairs. AR 361 FWD and REV primers were used to integrate a BssHII recognition site within the nucleotide sequence corresponding to amino acid 361 for constructs 361-392, 361-420, and 361-449. A similar approach was used for deletion constructs 361-392 and 392-420 using AR 392 FWD and REV primers. Deletion constructs 361-420, 392-420, and 420-449 used AR 420 FWD and REV. Deletion constructs 420-449, 449-490, and 361-449 used AR 449 FWD and REV. Deletion constructs 420-490 and 449-490 using AR 490 FWD and REV (Table 1). After integration of BssHII sites at two sites, plasmids were then cut with BssHII and re-ligated. Constructs containing regions 422-439 ,433-450, and 473-490 were generated by annealing oligonucleotides (Table 1) and phosphorylating double stranded DNA cassettes and ligating them into AR 1/2/3 CE3ΔTAU5 which was cut by BssHII.

Luciferase Reporter Gene Assays – LNCaP cells were transfected by electroporation exactly as described [34], [46]. In summary, LNCaP cells (1 T-175 suspended in 350ul RMPI 1640 + 10%CSS) were mixed with a total of 12ug of DNA (9ug of the MMTV or

-5746 PSA reporter, 3ug SV-40 Renilla, and .5ug of activator) or with 12ug DNA + 120pmol siRNA. Cells were electroporated at 305 V for 10 ms with 1 pulse (BTX Electro Square Porator ECM 830). After 15minute recovery time, cells were plated into a 24-well plate. 24 hours post-transfection cells were re-fed with serum-free medium containing 1nmol/L mibolerone or 0.1% (v/v) ethanol as vehicle control for 24 hours. Cells were harvested in 1x passive lysis buffer provided in a Dual Luciferase Assay kit (Promega).

DU145 cells were transfected using Superfect reagent (Qiagen) exactly as described [34]. In summary, DU145 cells were plated in 24 well plates at a density of 60,000 cells per well in DMEM+10%CSS. After 24 hours, cells were transfected with 450ng of DNA (300 ng reporter, 100 ng of SV-40 Renilla, and 50ng of AR construct) +2ul Superfect per well. After 3 hours, media was removed and new DMEM+10%CSS was added. After 24 hours cells were re-fed with serum-free medium containing 1nmol/L mibolerone or 0.1% (v/v) ethanol as vehicle control for 24 hours. Cells were harvested in 1x passive lysis buffer provided in a Dual Luciferase Assay kit (Promega).

Activities of the firefly and renilla luciferase reporters were assayed using a Dual Luciferase Assay kit as per the manufacturer's recommendations. Transfection efficiency was normalized by dividing firefly luciferase activity by renilla luciferase activity. Data presented represent the mean +/- S.E from at least three independent experiments, each performed in triplicate.

Subcellular Fractionation- LNCaP cells were electroporated under androgen-free conditions as described above. Cells were transfected with 2ug of AR construct and

10ug Bluescript plasmid. Transfected cells were cultured 24 h and then treated for 24h with medium containing 1 nmol/L mibolerone or 0.1% ethanol. After treatment, transfected cells were washed in 1x phosphate-buffered saline (PBS), harvested in hypotonic buffer (250 mM sucrose, 20 mM HEPES, pH 7.4, 10mm KCl, 1.5 mm $MgCl_2$, 1mm EDTA, 1mm EGTA, and a 1x final concentration of a complete protease inhibitor (Roche Applied Science) and incubated for 20min on ice. Cells were lysed by 10 passages through 25-guage needles. The cytosolic fraction (supernatant) was collected by centrifugation at 720 x g for 5 min at 4° C. The nuclear pellet was washed twice by resuspending in 500 ul of hypotonic buffer followed by 10 passages through 25-gauge needles. Nuclei were pelleted by centrifugation at 3000 X g for 10 min at 4° C. Isolated cytosolic and nuclear fractions were resuspended in 1x Laemmli buffer (125mM Tris-HCL, pH 6.8, 4% (w/v) SDS, 20% (v/v) glycerol, 10% (v/v) β-mercaptoethanol, 0.004% bromophenol blue) boiled for 5 min, and then loaded equally by volume for Western blot analysis.

Immunofluorescence- Cos-7 cells were seeded in complete medium on coverslips the day before transfection. Cells were transfected under androgen-free conditions with 1ug of expression vector encoding full- length AR or truncated AR variants using Lipofectamine 2000 (Invitrogen). The next day culture medium was replaced with serum-free medium contain 1 nmol/L mibolerone or 0.1% (v/v) ethanol. Cells were maintained an additional 24h and then fixed with ice-cold methanol for 10 min, permeablized with 0.2% Triton X-100 in PBS for 15 min at room temperature, and blocked with 1XPBS containing 10% FBS for 1h at room temperature. Cells were

incubated overnight at 4 C with AR- N20 diluted 1:1000. Unbound primary antibody was removed by three washes with PBS, and then cells were incubated with fluorescein isothiocyanate (FITC)-conjugated secondary antibody (Jackson ImmunoResearch Laboratories) at a 1:500 dilution in PBS containing 0.2ug/ml 4',6-diamidino-2-phenylindole (DAPI) for 1h at room temperature. Slides were washed with PBS and mounted with Mowoil (Calbiochem), and images were captured using a confocal laser scanning microscope (Olympus Fluoview FV500 equipped with a 40x objective). The exposure and gain for FITC were 300ms and 592 respectively and the exposure and gain for DAPI were 450ms and 15 respectively. These values remained consistent for all samples.

Western Blot- Cell lysates in 1 X Laemmli buffer were subjected to Western Blot analysis as described[34]. In summary, samples were run on 7.5% resolving SDS-polyacrylamide gels. Gels were transferred at 100V/350mA for 1hr at room temperature. Membranes were blocked in blocking buffer (1XTBST, 5%w/v non-fat dry milk) for 30mins at room temperature. Next, blots were incubated with primary antibodies overnight at 4°C at a concentration of 1:1000 in blocking buffer. Blots were then incubated with the appropriate horseradish peroxidase-conjugated secondary antibodies at a concentration of 1:10,000 at room temperature for 2hrs. Blots were developed by incubation with Super Signal chemiluminescence reagent (Pierce) and exposed to film.

Primer	Use	Sequence 5'>3'
AHTAA mut FWD	mutagenesis	GCCGCCGCTTCCTCATCCGCGCACACTGCCGCCACAGCCGAAGAAGGCC
AHTAA mut REV	mutagenesis	GGCCTTCTTCGGCTGTGGCGGCAGTCTGCGCGGATGAGGAGGCGGCGGC
AR 361 FWD	mutagenesis	CGAGGCAGCTGCGTACCAGGCGCGCGACTACTACAACTTTCCACTGG
AR 361 REV	mutagenesis	CCAGTGGAAAGTTGTAGTAGTCGCGCGCCTGGTACGCAGCTGCCTCG
AR 392 FWD	mutagenesis	CTGGAGAACCCGCTGGCGCGCGGCAGCGCCTGGGCGGC
AR 392 REV	mutagenesis	GCCGCCCAGGCGCTGCCGCGCGCCAGCGGGTTCTCCAG
AR 420 FWD	mutagenesis	CATGGCGCGGGTGCAGCGCGCCCCGGTTCTGGGTCAC
AR 420 REV	mutagenesis	GTGACCCAGAACCGGGGCGCGCTGCACCCGCGCCATG
AR 449 FWD	mutagenesis	GGCCAGTTGTATGGAGCGCGCGGTGGTGGTGGGGGTGG
AR 449 REV	mutagenesis	CCACCCCCACCACCACCGCGCGCTCCATACAACTGGCC
AR 490 FWD	mutagenesis	CGGCCCCCTCAGGGGCTGGCGCGCCAGGAAAGCGACTTCACC
AR 490 REV	mutagenesis	GGTGAAGTCGCTTTCCTGGCGCGCCAGCCCCTGAGGGGGCCG
442-439 FWD	synthetic cassette	CGCGCCCCGGTTCTGGGTCACCCTCAGCCGCCGCTTCCTCATCCTGGCACACTCTCTTCG
422-439 REV	synthetic cassette	CGCGCGAAGAGAGTGTGCCAGGATGAGGAAGCGGCGGCTGAGGGTGACCCAGAACCGGGG
433-450 FWD	synthetic cassette	CGCGCTCATCCTGGCACACTCTCTTCACAGCCGAAGAAGGCCAGTTGTATGGACCGTGTG
433-450 REV	synthetic cassette	CGCGCACACGGTCCATACAACTGGCCTTCTTCGGCTGTGAAGAGAGTGTGCCAGGATGAG
473-490 FWD	synthetic cassette	CGCGCGAGGCGGGAGCTGTAGCCCCCTACGGCTACACTCGGCCCCCTCAGGGGCTGG
473-490 REV	synthetic cassette	CGCGCCAGCCCCTGAGGGGGCCGAGTGTAGCCGTAGGGGGCTACAGCTCCGCGCTCG

Table 1

RESULTS

The TAU5 transcriptional activation domain has been shown to be required for aberrant AR activation in CRPC cells under conditions of no or low androgens [46]. To determine whether AR variants also require TAU5 for transcriptional activation we performed luciferase assays. AR-null DU145 cells were transfected with a 4XARE-luciferase reporter construct along with AR 1/2/3 CE3 or AR 1/2/3 CE3ΔTAU5 (Fig 2 A & B). AR was activated upon the addition mibolerone and AR 1/2/3 CE3 displayed constitutive activity. However, deletion of the TAU5 domain impaired transcriptional activity of the AR 1/2/3 CE3 variant. Immunoblots confirmed that protein level stayed consistent within the experiment. This loss of transcriptional activity suggested that AR 1/2/3 CE3 is dependent on the TAU5 domain for transcription.

To address whether additional AR splice variants which contain unique c-terminal tails may also be dependent on the AR TAU5 domain for transcriptional activity, the same assays were performed with AR 1/2/3 2b (Fig 2C). DU145 cells were transfected with the reporter construct 4xARE, AR, AR 1/2/3 2b, or AR 1/2/3 2bΔTAU5. As expected, AR was activated upon the addition of androgens and AR 1/2/3 2b was constituvely active. However, AR 1/2/3 2bΔTAU5 displayed significantly lower transcriptional activity. Immunoblots confirmed that protein level stayed consistent. This loss of transcriptional activity suggested that AR 1/2/3 2b is dependent on the TAU5 domain for transcription.

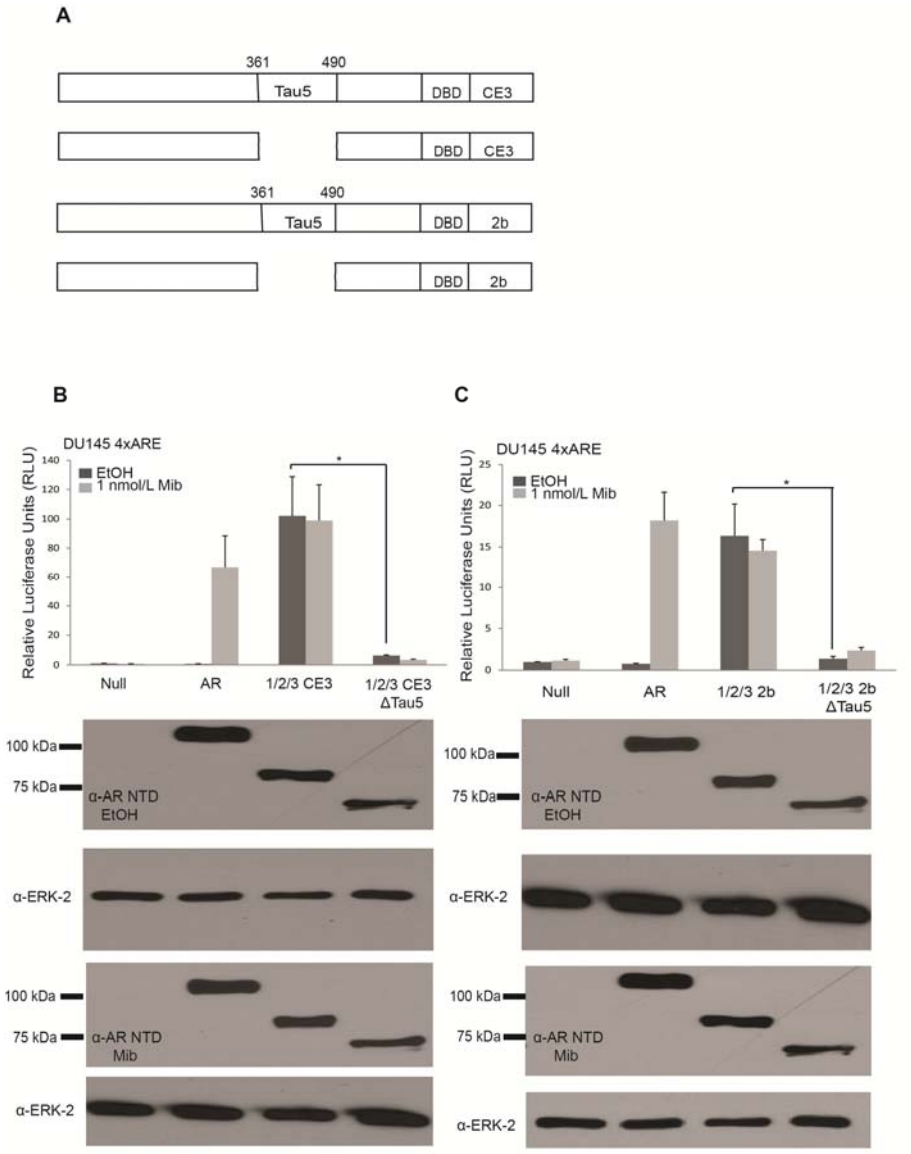
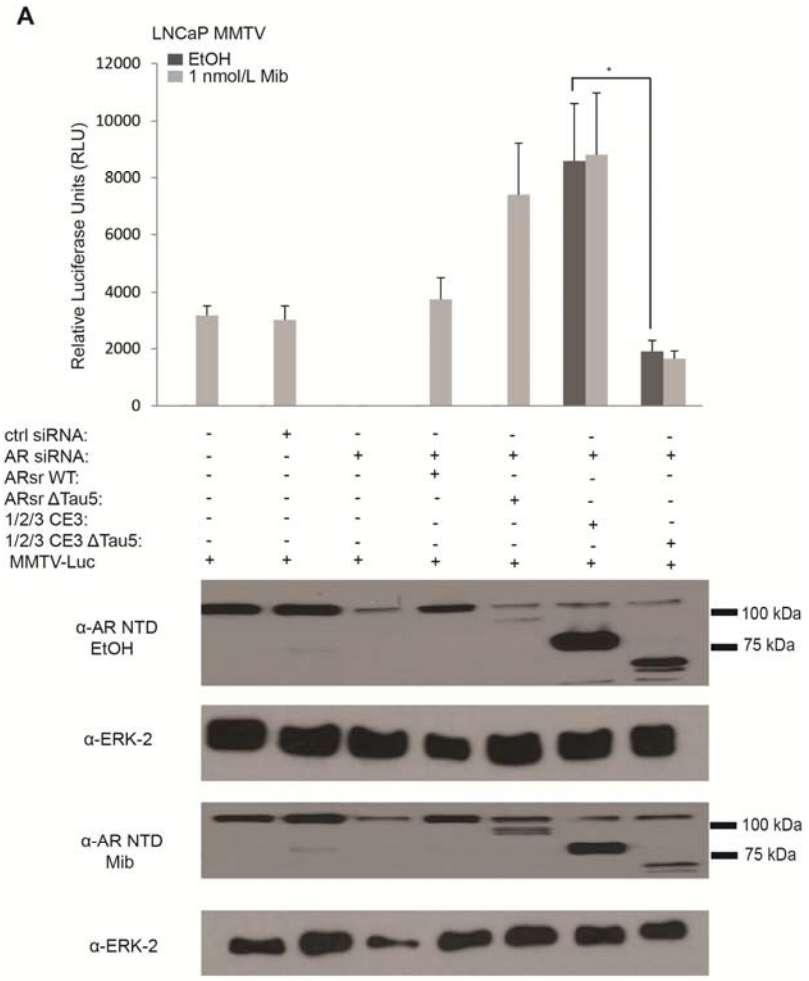


Figure 2: Truncated AR variants are dependent on TAU5 for transcriptional activity
A: AR 1/2/3 CE3 and 1/2/3 2b constructs with TAU5 were deleted to generate: AR 1/2/3 CE3ΔTAU5 and 1/2/3 2bΔTAU5.
B: Luciferase assay in DU145 cells. Cells were transfected with a 4xARE-luciferase reporter and AR expression constructs as indicated. Cells were treated with ethanol (EtOH) or 1nmol/L mibolerone (Mib). Columns represent the average of three separate experiments, each performed in triplicate; bars, SE. *P=.006. Western blots were performed with lysates from transfected cells, and probed using antibodies specific for AR or ERK-2 (loading control).C: Experiments were performed exactly as in 2B except AR 1/2/3 2b constructs were transfected into cells. *P=.002.

To verify TAU5 was necessary for full transcriptional activity of the AR variants but not full length AR and to verify this finding was not restricted to one cell line, a knockdown/rescue experiment was performed in LNCaP cells. LNCaP cells were transfected with an MMTV-luciferase reporter construct, along with control siRNA or AR targeted siRNA (Fig 3). AR knock-down was rescued by co-transfection with siRNA resistant forms of full length AR, ARΔTAU5, AR 1/2/3 CE3, or 1/2/3 CE3ΔTAU5 (Fig 3A). AR knockdown resulted in loss of androgen-dependent transcriptional activation. Addition of siRNA resistant AR rescued this androgen-dependent transcriptional activity. However, when the AR knock-down was rescued with siRNA-resistant AR 1/2/3 CE3, constitutive activation of the reporter was observed. As shown previously, deletion of TAU5 from full-length AR resulted in a significant increase in androgen-mediated transcriptional activity [46]. In contrast, deletion of TAU5 from AR 1/2/3 CE3 significantly impaired constitutive transcriptional activity. Immunoblots were performed and confirmed the expected patterns of AR knock-down and that protein expression remained consistent. These data suggest that TAU5 region plays an important activating role in transcription mediated by truncated AR variants, but not full length AR.

A similar set of knock-down/rescue experiments were performed with a reporter construct based on the PSA promoter/enhancer fragment (Fig 3B). As expected, in the untreated conditions or in the presence of control siRNA the addition of androgens resulted in activation of endogenous AR. AR knockdown resulted in loss of transcriptional activation and addition of siRNA resistant AR rescued this transcriptional activity. AR 1/2/3 CE3 and AR 1/2/3 2b remained constituvely active.

ARΔTAU5 showed an increase in transcriptional activity. In contrast, AR 1/2/3 CE3ΔTAU5 and AR 1/2/3 2bΔTAU5 had a significant decrease in transcriptional activity. Immunoblots were performed and confirmed expected patterns of AR knockdown as well as that protein expression levels remained consistent. These data further support the novel concept that the TAU5 region plays an inhibitory role for full length AR but an activating role in supporting constitutive transcriptional activity of truncated AR variants.



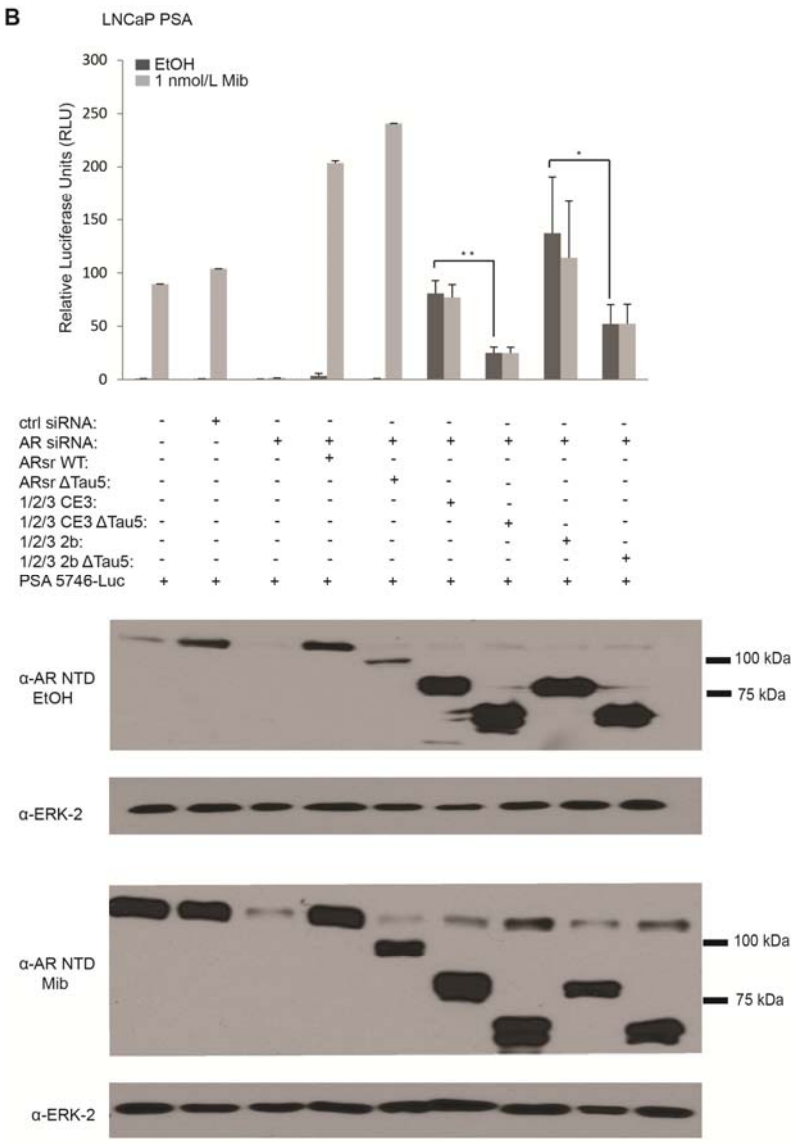
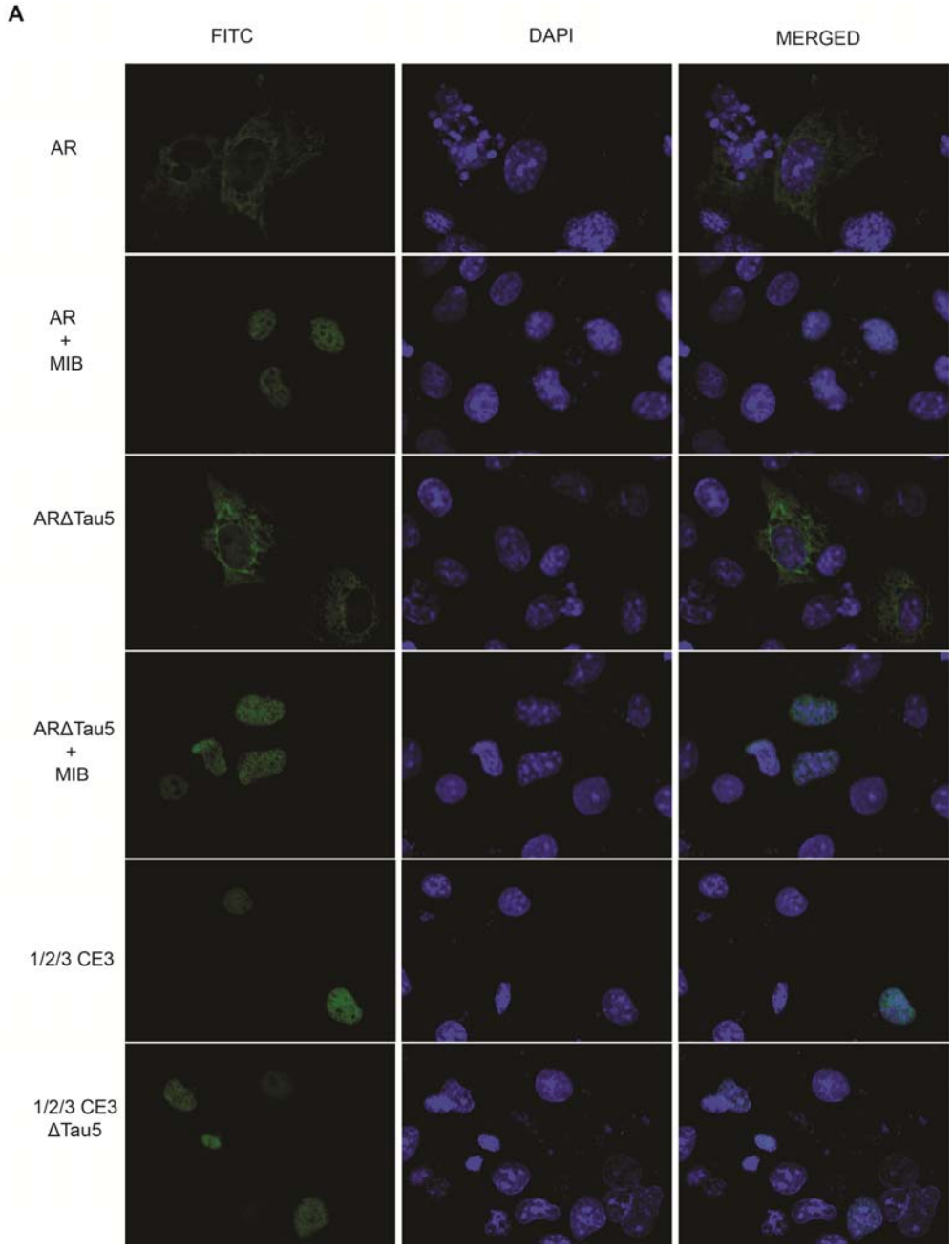


Figure 3: Differential TAU5 requirement for transcription mediated by truncated AR variants vs. full-length AR.
A: Luciferase assay in LNCaP cells. Cells were transfected with a MMTV-luciferase reporter. Endogenous AR was knocked down using a siRNA targeting exon 1. siRNA resistant constructs were co-transfected as indicated. Cells were treated with ethanol (EtOH) or 1nmol/L mibolerone (Mib). Columns represent the average of three separate experiments, each performed in triplicate; bars, SE. *P=.005 Western blots were performed with lysates from transfected cells, and probed using antibodies specific for AR or ERK-2 (loading control).
B: Experiments were performed exactly as in 3A but -5746 PSA- luciferase was used as a reporter and both AR 1/2/3 CE3 and 1/2/3 2b constructs were transfected into cells. *P=.05 and **P=$1x10^{-5}$

To address whether the TAU5 domain was playing a dichotomous role in regulating transcriptional activity of truncated AR variants, and was not merely inhibiting upstream regulatory mechanisms such as nuclear localization, we assessed nuclear localization of 1/2/3/CE3 and AR 1/2/3 CE3ΔTAU5 (Fig 4). The AR-null Cos7 cell line was transfected with various AR constructs and their localization was visualized by fluorescence microscopy (Fig 4A). Under androgen-free conditions, AR localized to the cytoplasm, and in the presence of androgens AR localized to the nucleus. Deletion of TAU5 did not impair this androgen-dependent localization pattern for full-length AR. Consistent with the constitutive transcriptional activity observed in the absence of androgens, AR 1/2/3 CE3 localized to the nucleus in an androgen-independent manner. Similarly, in non-androgen conditions AR 1/2/3 CE3ΔTAU5 localized to the nucleus. These data indicate that the loss of the TAU5 regions does not impair localization and therefore further supports TAU5 is an important AR transcriptional activation domain.

To further verify that localization of truncated AR variants was not affected by deletion of the TAU5 domain, biochemical fractionation was employed (Fig 4B-D). LNCaP cells were transfected with various AR expression vectors and then nuclear and cytoplasmic compartments were separated by subcellular fractionation. In line with immunofluorescence data, AR and ARΔTAU5 displayed predominantly cytoplasmic localization. Upon addition of androgens, these proteins localized efficiently to the nucleus. Conversely, under androgen-free conditions AR 1/2/3 CE3 localized constitutively to the nucleus. Deletion of the TAU5 domain from AR 1/2/3/CE3 did not impair this nuclear localization. The biochemical data supports the microscopy data in

that the loss of the TAU5 regions does not impair localization and therefore further supports TAU5 as an important AR transcriptional activation domain.



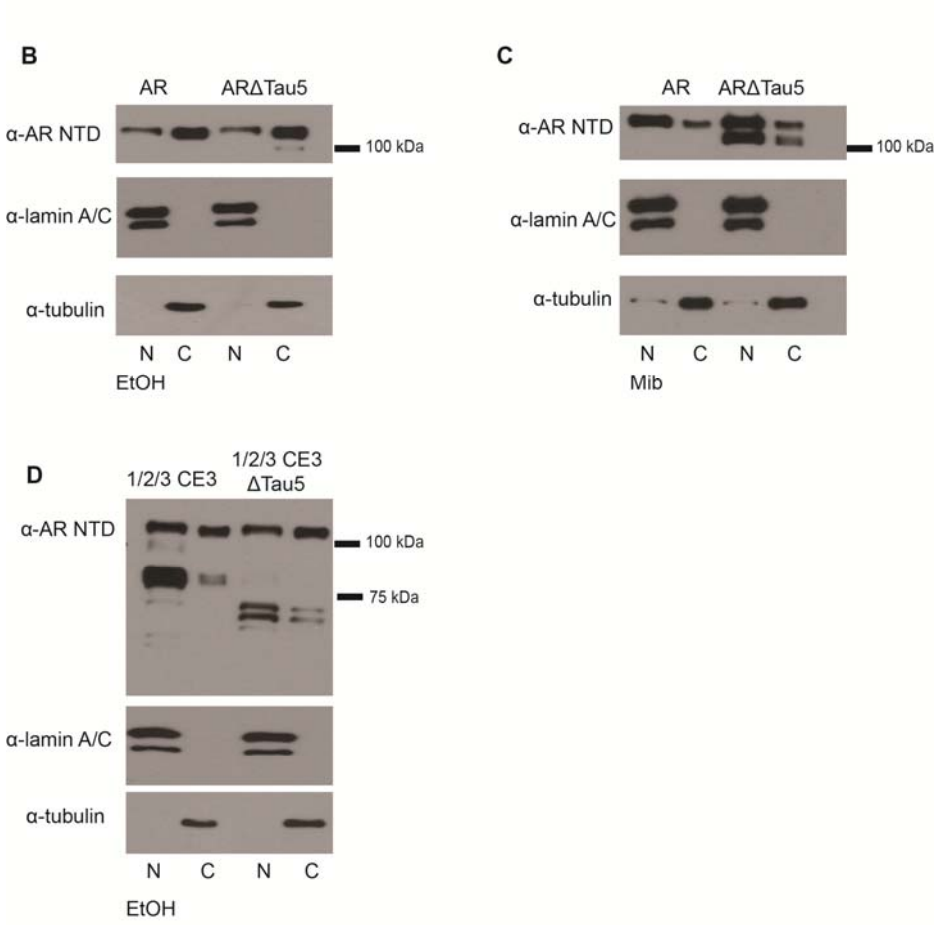


Figure 4: AR variants localize to nucleus independent of TAU5
A: Cos-7 cells were transfected with various AR expression constructs and stained with antibody specific for the NTD (FITC signal), and nuclei were stained with DAPI. Stained cells were visualized by confocal microscopy. Representative images are shown for three color channels (FITC, DAPI, and merged FITC/DAPI).
B,C,D: LNCaP cells expressing AR constructs were cultured in serum free-medium containing 1nmol/L Mibolerone (Mib) or ethanol (EtOH). Cell lysates were separated into nuclear (N) and cytoplasmic (C) fractions and analyzed by Western blot with antibodies specific for AR NTD, lamin A/C (nuclear marker), and tubulin (cytoplasmic marker).

Within the TAU5 domain, the WHTLF motif has been identified as an amino acid sequence required for AR ligand-independent activity in no or low androgen conditions [46]. To determine whether WHTLF was also required for AR variant transcriptional activity, AR mutants were generated in which the WHTLF motif was mutated to AHTAA (AR 1/2/3 CE3 AHTAA and AR 1/2/3 2b AHTAA) (Fig 5A). In the first set of experiments, LNCaP cells were then transfected with MMTV-luciferase (Fig 5B) or -5746 PSA-luciferase (Fig 5D) and AR 1/2/3 CE3, 1/2/CE3ΔTAU5 or AR 1/2/3 CE3 AHTAA. AR 1/2/3 CE3 remained constitutively active while AR 1/2/3 CE3ΔTAU5 had significantly lower transcriptional activity. AR 1/2/3 CE3 AHTAA transcription levels were similar to AR 1/2/3 CE3. Immunoblots confirmed similar levels of protein present in the experiment. Therefore for 1/2/3/CE3, transcriptional activity is dependent on TAU5 but not the WHTLF motif.

In the second set of experiments, LNCaP cells were transfected with MMTV and AR 1/2/3 2b, AR 1/2/3 2bΔTAU5, or AR 1/2/3 2b AHTAA (Fig 5C). AR 1/2/3 2b remained constitutively active while AR 1/2/3 2bΔTAU5 had significantly lower transcriptional activity. AR 1/2/3 2b AHTAA transcription levels were similar to AR 1/2/3 2b. Immunoblots confirmed similar levels of protein present in the experiment. Based on these data, we conclude that for both AR variants transcriptional activity is dependent on TAU5 but not the WHTLF motif.

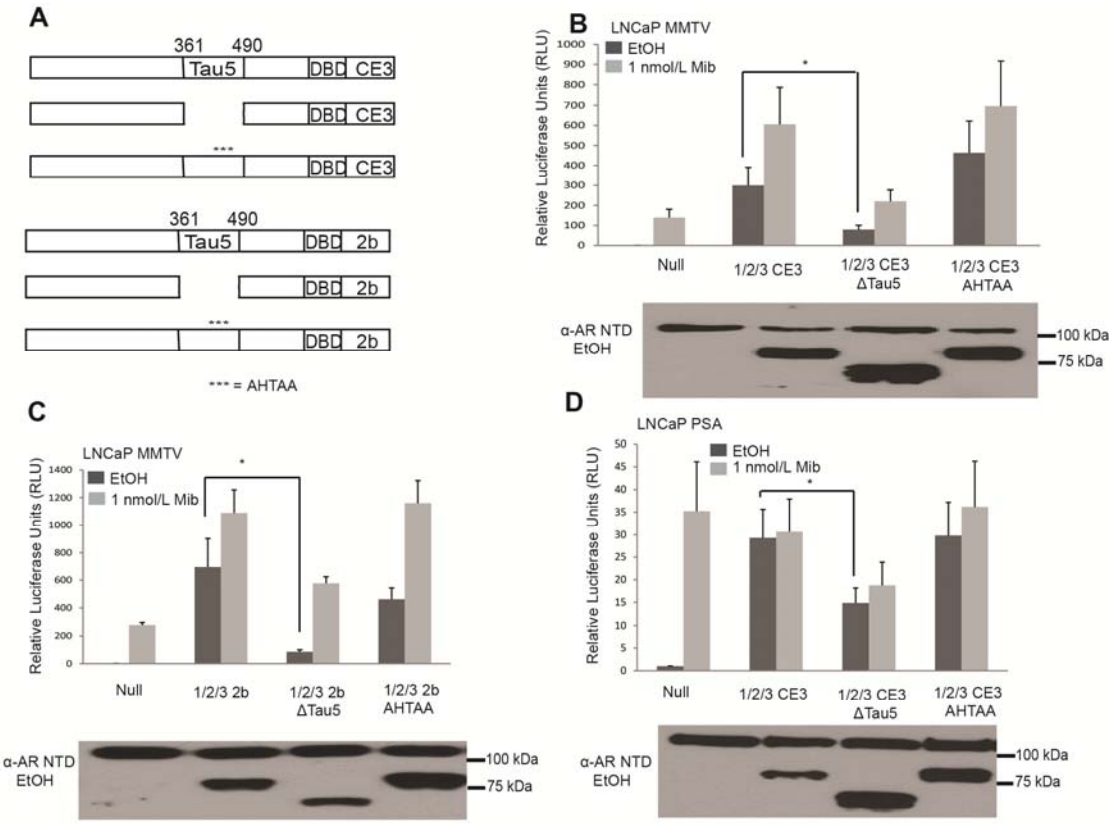


Figure 5: Truncated AR variants do not require the WHTLF transactivation motif for ligand-independent transcriptional Activity
A: AR 1/2/3 CE3 and 1/2/3 2b were mutated to generate 1/2/3 CE3 AHTAA and 1/2/3 2b AHTAA marked by the ***.
B: Luciferase assay in LNCaP cells. Cells were transfected with a MMTV-luciferase reporter and AR expression constructs as indicated. Cells were treated with ethanol (EtOH) or 1nmol/L mibolerone (Mib). Columns represent are an average of three separate experiments, each performed in triplicate; bars, SE.*P=.004. Western blots were performed with lysates from transfected cells, and probed using antibodies specific for AR or ERK-2 (loading control).
C: Same as 4B but AR 1/2/3 2b constructs were transfected into cells. *P=.02
D: Same as 4B but -5746 PSA- luciferase is the reporter. *P=.009

Having determined that TAU5 was important for constitutive transcriptional activity of truncated AR variants, but that the function of the WHTLF transcriptional activation motif was dispensable, we tested which region of TAU5 may be supporting transcriptional activity of truncated AR variants. TAU5 was divided into four regions 361-392, 392-420, 420-449, and 449-490 (Fig 6). Various combinations of these regions were deleted from the AR variants and transcriptional activity was measured. Deletion of 361-392, 361-420, 392-420, 420-449,449-490, and 361-449 did not result in significant changes in transcription levels compared to AR 1/2/3 CE3. However, deletion of 420-490 resulted in a significant decrease in transcription compared to AR 1/2/3 CE3 and had similar levels of transcription compared to AR 1/2/3 CE3ΔTAU5. Based on significant decrease in transcriptional activity there may be a motif between amino acids 420-490 that is responsible for TAU5 dependent transcriptional activity of truncated AR variants.

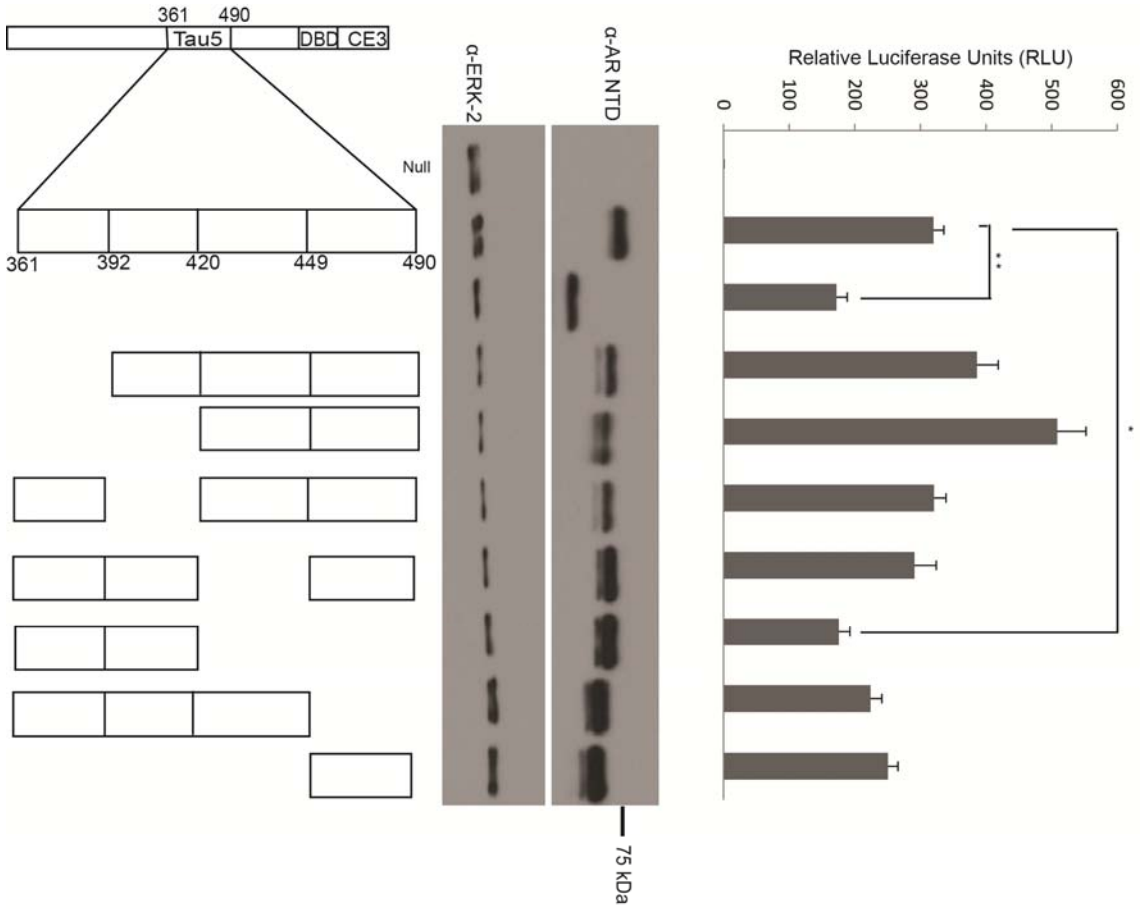


Figure 6: Deletional Analysis of TAU5 Luciferase Activity in AR 1/2/3 CE3 Luciferase assay in DU145 cells. Cells were transfected with a 4xARE-luciferase reporter and AR 1/2/3 CE3 deletion constructs as indicated. Boxes represent regions present in TAU5. Columns represent are an average of three separate experiments, each performed in triplicate; bars, SE. *P=$2x10^{-6}$ and **P=$3x10^{-13}$. Western blots were performed with lysates from transfected cells, and probed using antibodies specific for AR or ERK-2 (loading control).

To further narrow down the region between 420-490 that may be responsible for TAU5 dependent transcriptional activity of truncated AR variants, smaller peptide fragments within the deleted 420-490 region were added back to AR 1/2/3 CE3ΔTAU5. Amino acids 422-439, 432-450, and 473-490 were added back into the AR 1/2/3 CE3ΔTAU5 constructs (Fig 7). These expression vectors were then transfected into DU145 and transcriptional activity was measured. All three mutants had lower transcriptional activity than AR 1/2/3 CE3 but not as low as AR 1/2/3 CE3ΔTAU5. Therefore, the region important for TAU5 dependent transcription may be within 420-490 but the exact region or motif is unknown.

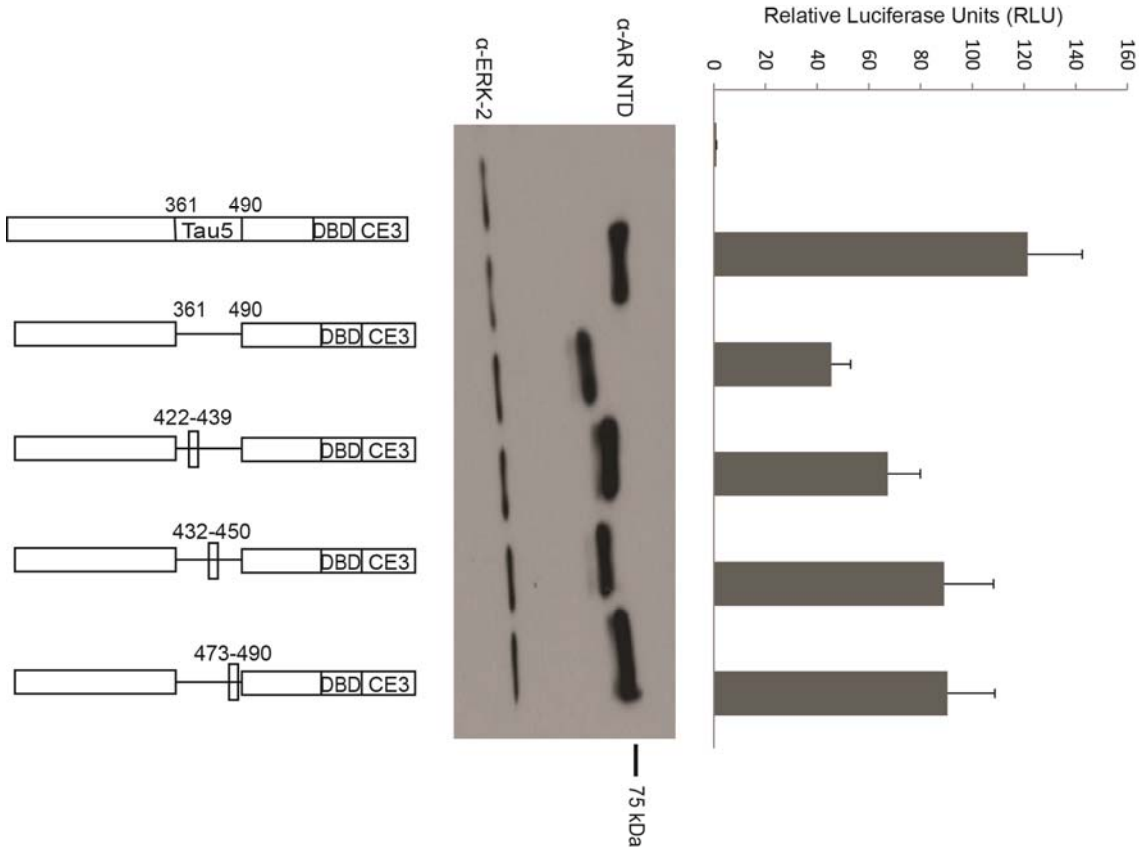


Figure 7: Insertional Analysis of TAU5 Luciferase Activity in AR 1/2/3 CE3 Luciferase assay in DU145 cells. Cells were transfected with a 4xARE-luciferase reporter and AR expression constructs as indicated. Columns represent are an average of three separate experiments, each performed in triplicate; bars, SE. Western blots were performed with lysates from transfected cells, and probed using antibodies specific for AR or ERK-2 (loading control).

DISCUSSION

In this project, we determined that the constitutive, ligand independent transcriptional activity of truncated AR variants AR 1/2/3 2b and AR 1/2/3 CE3 was dependent on TAU5 for transcription. Surprisingly, we found that the variants do not require WHTLF to mediate this ligand independent activity. We attempted to narrow down the region that may be important for variant transcriptional activity and found that deletion of amino acids 420-490 resulted in significantly lower activity compared to AR 1/2/3 CE3. However, testing smaller regions of 420-490 did not elucidate a specific motif.

The 420-490 region still contains WHTLF. The WHTLF motif is located in a region of predicted secondary structure including a predicated β-sheet at A430 and α-helices at E442 and E433[46]. The WHTLF motif itself is predicated to have secondary structure, adopting a helical conformation and the amino acids W435, L438, and F439 form a hydrophobic surface [46]. In this study, we found that mutation of these amino acids to alanine did not alter transcriptional activity of truncated AR variants. However, it is possible that the WHTLF motif plays a role in supporting structural dynamics of the NTD, and may play an important role in providing flanking regions with the support to form a proper secondary structure necessary for maximal transcriptional activity. One method to test this would be to delete WHTLF from the AR variants and then test the transcriptional activity.

As previously shown, the variants are constitutively active and localize in the nucleus. In this study, we confirmed that AR 1/2/3 CE3ΔTAU5 also localized in the nucleus. Therefore, the loss in transcriptional activity in the absence of TAU5 was not

due to an inability to localize in the nucleus. Localization data was shown both by immunofluorescence and subcellular fractionation. The molecular basis for the differences in transcriptional activity between AR 1/2/3 CE3 and 1/2/3 CE3ΔTAU5 may reside in various factors and below we discuss possible mechanisms of actions in depth.

AR COREGULATORS

Since the AR variant NTD is highly dynamic and does not contain a LBD, the AR variants do not form a NTD-CTD (N-C) interaction. Thus the 3-dimensional structure of the variants is fundamentally different from the full length AR. This difference underlies possible changes in activity between the AR and AR variants. For example, one mechanism to explain the differences between TAU5 transcriptional activation in the AR and in AR variants is by differential interactions with coregulators. Changes in structure could account for changes in coregulator recruitment. AR coregulators have been extensively reviewed previously [48], [49]. Of the coregulators discovered only two are known to bind specifically to TAU5.

SRC1

Steroid receptor co-activator 1 (SRC1) was the first nuclear hormone receptor cloned and characterized. Since the initial SRC1 discovery, two related family members were identified: SRC2 and SRC3 [50]. The SRC proteins interact with nuclear hormone receptors and are known to enhance their transcriptional activity [50]. SRC proteins are known to perform this function through several mechanisms. First, SRC1 and SRC3 can directly bind AR [49]. Second, SRC proteins form scaffolds by which

histone modifying proteins such as CBP/p300 can be recruited [49]. SRCs have a basic-helix-loop-Per/ARNT/Sim (bHLH-PAS) domain that is responsible for interacting with transcription factors and enhancing transcription [50]. SRC1 is known to bind to the AR at amino acids 360-494 (TAU5) as well as 625-919 [51], [52]. SRC is known to be overexpressed in advanced stages of prostate cancer [50]. It has been proposed that SRC1 enhances ligand independent AR activity via IL-6 and the MAPK pathway [53]. It has also been shown that SRC inhibition in androgen independent environments lowered AR transactivation potential [54]. The WHTLF motif in the AR has been shown to bind to the LBD enhancing an N-C interaction. This interaction slowed the disassociation rate of androgen [55]. One hypothesis is that without a LBD, without the requirement for an N-C interaction, and without bound androgens, SRC1 is able to interact with the AR variants NTD via TAU5 in a manner that does not require WHTLF.

DAXX

Daxx was originally identified as a cytoplasmic signaling molecule linking Jun N-terminal kinase in Fas-mediated apoptosis [56]. It interacts with the AR between amino acids 1-560 as well as 486-651 [57]. In addition, Daxx inhibits transcription of several transcription factors: ETS1, Pax3, glucocorticoid receptor, p53 family of proteins, and the mineralocorticoid receptor [57]. Furthermore, Daxx has also shown transcriptional co-regulator activity, for example Daxx was shown to have both coactivator or corepressor activity on transcriptional activation of the Pax5 gene depending on the cellular context [58]. Li et al showed that overexpression of Daxx

repressed AR activity and this repression was sumoylation dependent. Mutation of SUMO-conjugated sites resulted in loss of Daxx interaction [57]. One hypothesis could be that Daxx is recruited as a repressor for the full length AR but not for truncated AR variants. In this case, deletion of TAU5 would eliminate the sumoylation site from full length AR. The change in sumoylation could block any recruitment and/or activity of Daxx and thereby result in increased AR transcriptional activity. Because deletion of TAU5 impairs transcriptional activity of truncated AR variants, this indicates that these AR species may not be not be inhibited by sumoylation or Daxx activity. The change in sumoylation status may permit interactions with other cofactors through this domain, including SRC1.

AR POST-TRANSLATIONAL MODIFICATIONS

Phosphorylation

Post-translational modifications (PTM) play an important role in the activity of steroid receptors. PTM affect the receptors' stability, subcellular localization, and protein-protein interactions [59]. The AR is phosphorylated at several sites with two sites found in the TAU5 region: Y363 and S424 [59–62]. In this study we generated smaller deletions of TAU5 and tested the transcriptional activity of these constructs. We found that deletion of 420-490 resulted in transcriptional activity similar to deletion of the entire TAU5 region. Based on this data, the S424 site may be important for regulation of truncated AR variant transcriptional activity.

The function of AR phosphorylation of either site is yet unknown. The data available for Y363 may be a clue to the regulation of S424. It was recently shown that in 8 out of 18 CRPC samples there was upregulation of tyrosine phosphorylation of AR

at Y363 [62]. This phosphorylation was associated with the activated cdc42 -associated kinase 1 (Ack1) activation. Mutation of Y363 resulted in reduced Ack1 mediated AR reporter activation as well as abrogated recruitment to the androgen-responsive PSA enhancer [62]. The current model proposed is that Ack1 is autophosphorylated by autocrine or paracrine factors, or by mutation. This activation recruits AR to the AREs and recruits other cofactors to allow for gene expression in low androgen conditions [62]. One hypothesis is that the variants are phosphorylated at the S424 site. Loss of TAU5 results in loss of the phosphorylation and coactivator recruitment necessary for constitutive activity.

Sumoylation

Sumoylation is the process of adding a Small Ubiquitin-like Modifier (SUMO) to a protein [63]. This process occurs by an E1 activating enzyme, E2 conjugation enzyme, and E3 ligase [63]. Sumoylation is a highly conserved process that can have significant effects on the cells. It can alter subcellular localization of a protein, DNA-binding or transcriptional potential, alter the MAP kinase pathway, as well as have repressive effects on transcription factors [59]. The AR is sumoylated at two sites: predominately at K386 and to a lesser extent at K520 [64]. It has been shown that sumoylation of these sites in the NTD results in mainly repression of the AR [65]. Sumoylation also appears to be hormone dependent, unliganded or antagonist bound AR has been shown to unsumoylated [65]. One hypothesis is that deletion of TAU5 from full length AR removes the sumoylation related repression. Furthermore, the constitutively active variants are not sumoylated because they lack a CTD to bind

hormone. Therefore deletion of TAU5 in truncated AR variants may result in the loss of a unknown activating cofactor that is recruited independent of the sumoylation status. Below is a summary of the possible future directions (Table 2).

Summary of Future Directions		Relation to TAU5
AR Coregulators	SRC1	Interacts with TAU5 and the WHTLF motif [51].
	DAXX	Interacts with TAU5 and recruitment is sumoylation dependent [57].
Post-translational Modifications	Phosphorylation	Phosphorylation sites at Y363 and S424 in TAU5 [61].
	Sumoylation	Sumoylation sites at K386 (in TAU5) and K520 [64].

CONCLUSION

In conclusion, this study showed that the full length AR and AR variants rely on different domain in the NTD for transcriptional activity. This study is important because the current state of prostate cancer therapy rationally targets the androgen receptor. MDV3100 is a rationally designed drug that targets the LBD. It prevents AR nuclear translocation, DNA binding, and AR coactivator recruitment leading to the apoptosis of cells [27]. Recently, truncated AR variants were demonstrated to mediate

resistance to MDV3100 and other drugs that bind to the AR LBD [66]. This study makes the case that drugs targeting the AR NTD should be pursued because these drugs would block activity of truncated AR variants. This has been previously shown by the use of AR NTD_{1-558} decoy molecules that blocked full length AR activity by presumably competing for coregulators. More recently, EPI-001 was identified as a drug targeting the NTD of the AR, and was shown to inhibit AR activity in the absence of ligand [67], [68]. This study shows that rationally designing a drug against the NTD requires mechanistic understanding of activation dynamics for both the full length AR and truncated AR variants. For example, the data outlined in this thesis indicate that a drug targeting TAU5 may unintentionally hyperactivate full length AR while inhibiting truncated AR variants, which could promote disease progression. The findings from this study lay important groundwork for understanding the mechanisms underlying the role for TAU5 in supporting activity of truncated AR variants. Ultimately, this knowledge could lead to the development of more effective AR-targeted drugs to treat CRPC.

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