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13. ABSTRACT (Maximum 200 Words)

Previously we discovered that a modest increase in androgen receptor (AR) expression is sufficient and necessary for hormone refractory (HR) prostate cancer progression. Together with published results of common AR overexpression in HR clinical tumor samples, our results indicate that AR overexpression is a cause for HR disease. We also demonstrated that the canonical transcriptional activity of AR is required in this process. Since the AR transcriptional function is mediated by nuclear receptor cofactors, we proposed to study their role in the HR progression. In the first year of study, we proposed to determine if chromatin remodeling or other functions of nuclear receptor cofactors are involved in the HR progression and if coactivators and corepressors are critical in this process. We unexpectedly discovered that blocking deacetylase activities inhibited AR activity, measured by endogenous PSA expression. This is in contrast to published data that deacetylase activities are transcriptionally inhibitory in exogenous reporter systems. We also demonstrated that TRβ had no squelching effect on AR transcriptional activity, measured by endogenous PSA expression. Finally, we had developed ChIP assay to detect AR and Pol II associated with the PSA promoter

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INTRODUCTION

Previously we discovered that a modest increase in androgen receptor (AR) expression is sufficient and necessary for hormone refractory (HR) prostate cancer progression. Together with published results of common AR overexpression in HR clinical tumor samples, our results indicate that AR overexpression is a cause for HR disease. We also demonstrated that the canonical transcriptional activity of AR is required in this process. Since the AR transcriptional function is mediated by nuclear receptor cofactors, we proposed to study their role in the HR progression.

BODY

Task 1. To determine if chromatin remodeling or other functions of nuclear receptor cofactors are involved in the HR progression and if coactivators or corepressors are critical in this process.

1.1 To determine if chromatin remodeling or other functions of nuclear receptor cofactors are involved

We have generated AR-overexpressed LNCaP cells (LN/AR) by a lentivirus system. AR expression in LN/AR cells was about 3-fold higher than in its parental cells (Figure 1, lane 3 to 1). Over-expressed AR maintained the property of the endogenous one because AR in LN/AR cells was also stabilized when a synthetic androgen R1881 was present (Figure 1, lane 2 to 1, and lane 4 to 3).

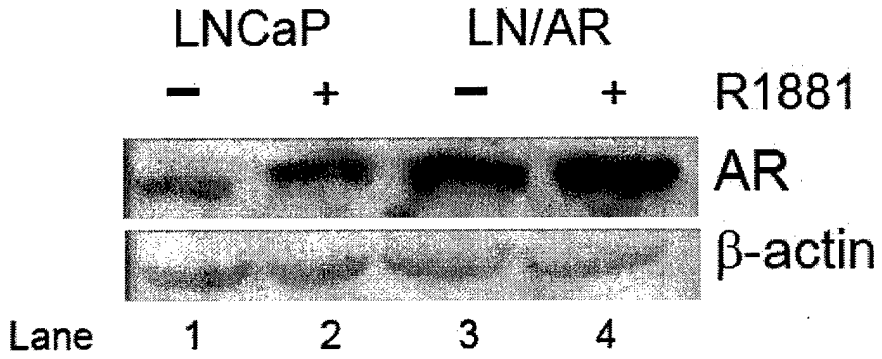


Figure 1. Engineered AR-overexpressed LNCaP cells by a lentivirus system. Western blot analysis of AR protein levels in LNCaP (lanes 1 and 2) or LN/AR (lanes 3 and 4) in the absence (lanes 1 and 3) or presence of 1 nM of R1881 (lanes 2 and 4).

We hypothesized that TSA treatment will cause hormone sensitive LNCaP cells to become HR if deacetylase activities are responsible for preventing HR progression. To examine this hypothesis, LNCaP cells were treated with increasing doses of trichostatin A (TSA) in the absence or presence of R1881, and PSA expression was measured by

western blot analysis as the readout for AR activity. In contrast to published reports, low doses (0.01 and 0.1 microM) of TSA treatment did not affect PSA expression because these doses did not cause accumulation of acetyl-histone 3 (Ac-H3) or α-tubulin acetylation (Ac-α-Tubulin) (Figure 2a, lanes 1-3 and 5-7). LNCaP cells treated with 1 μM of TSA inhibited deacetylase activities as demonstrated by accumulation of Ac-H3 and Ac-α-Tubulin (Figure 2a, lanes 4 and 8). However, this treatment surprisingly inhibited PSA expression, which is completely opposite to the reported data that blocking deacetylase activities enhances AR transcription in exogenous systems. The inhibition of TSA on PSA production was confirmed by ELISA on secreted PSA (Figure 2b).

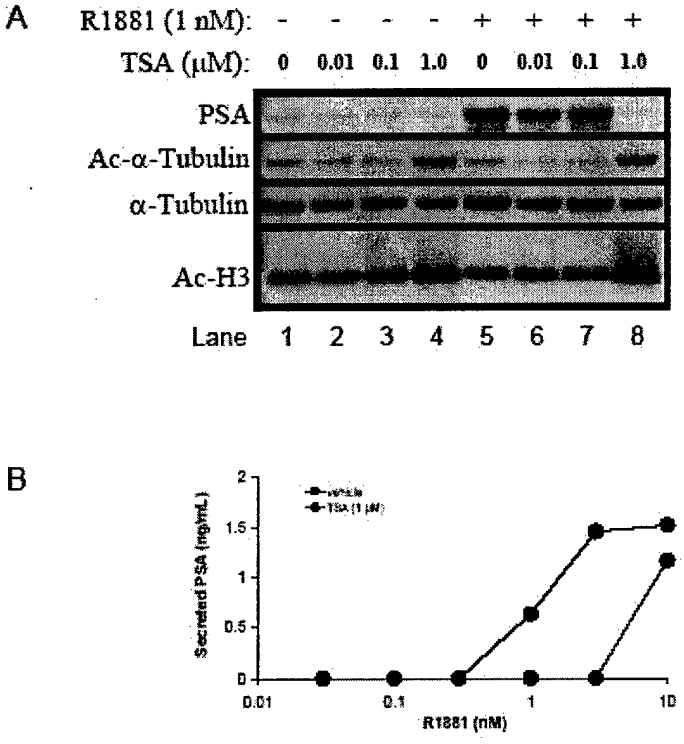


Figure 2. TSA inhibited AR transcriptional activity. A, Western blot analysis of PSA, Ac-α-tubulin, α-tubulin, and Ac-H3 levels in the absence (lanes 1-4) or presence (lanes 5-8) of 1 nM of R1881 in LNCaP cells treated with different concentrations of TSA. B, Secreted PSA levels in LNCaP cells treated with vehicle or 1 μM of TSA with increasing concentrations of R1881.

To determine if TSA-mediated inhibition of PSA is due to the endogenous native chromatin structure, we performed a reporter assay using a luciferase reporter controlled by four tandem repeats of AR-responsive elements (4ARE). Consistent with reported data, TSA treatment augmented the transcriptional activities of AR on the exogenous episomal reporter (Figure 3).

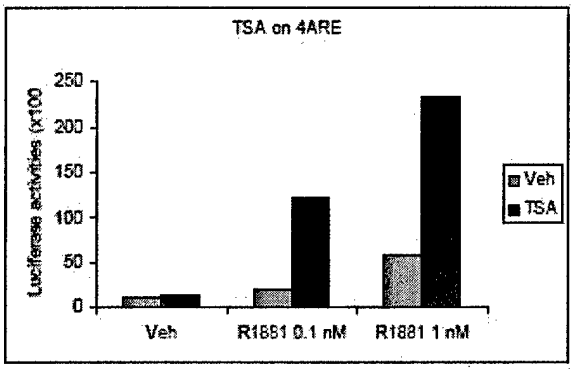


Figure 3. TSA stimulated AR-driven luciferase reporter.

To determine if the inhibitory effect is peculiar to TSA, we treated LNCaP cells with another deacetylase inhibitor, sodium butyrate. Sodium butyrate also inhibited PSA expression, measured by western blot analysis (Figure 4). These results indicated that blocking deacetylase activities did not render hormone refractory phenotype and that deacetylase activities are required for AR transcriptional activity on endogenous genes.

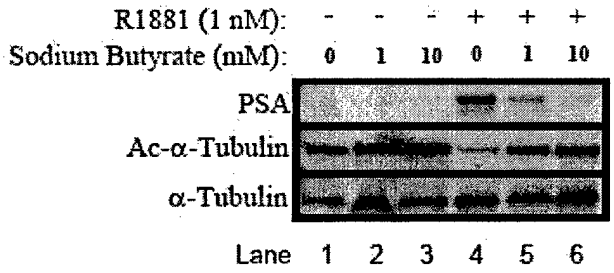


Figure 4. Sodium butyrate inhibited AR transcriptional activity on an endogenous gene. Western blot analysis of PSA, Ac-α-Tubulin and α-Tubulin in the absence (lanes 1-3) or presence (lanes 4-6) of 1 nM of R1881 with different concentrations of sodium butyrate.

1.2 To determine if coactivators or corepressors are critical in this process (4-7 months)

We have generated TRβ overexpression in LNCaP and LN/AR cells by a lentivirus system. TRβ expression in overexpressed lines are about 3-fold higher than its parental cells (Figure 5).

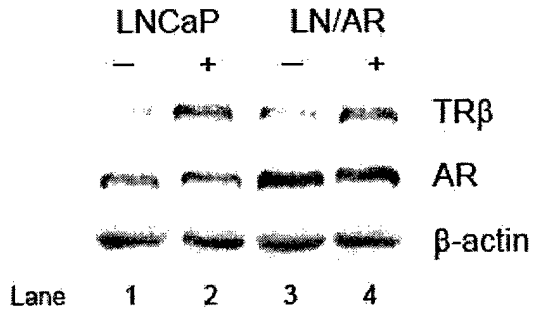
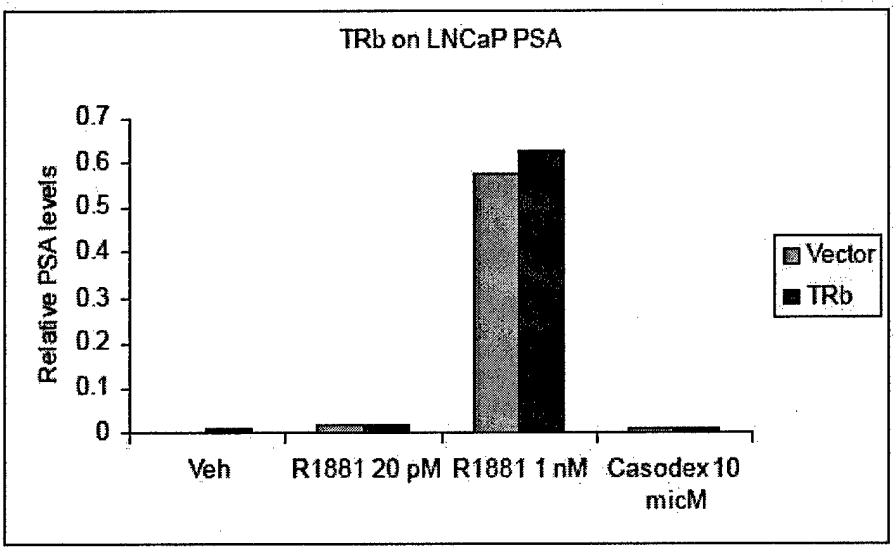


Figure 5. Engineered TRβ-overexpressed LNCaP cells by a lentivirus system. Western blot analysis of TRβ and AR protein levels in LNCaP (lanes 1 and 2) and LN/AR cells (lanes 3 and 4).

We hypothesized that overexpressed TRβ will titrate away corepressors and confer HR phenotype if the same corepressors for inhibiting AR activity are limited in prostate cancer. To test this hypothesis, we treated control and TRβ overexpressed LNCaP cells with different doses of R1881 or with AR transcriptional inhibitor Casodex, and measured secreted PSA production by ELISA. AR overexpression stimulated PSA production in low concentration of R1881 (20 pM) (Figure 6b to 6a, vector). TRβ overexpression did not significantly stimulate PSA production in LNCaP cells (Figure 6a). Also, TRβ overexpression did not result in de-repression of Casodex inhibition in LNCaP cells, but AR overexpression did (Figure 6b to 6a, vector). These data suggest that either corepressors are not limited factors in the progression of prostate cancer, or TRβ and AR do not use the same corepressors.

A



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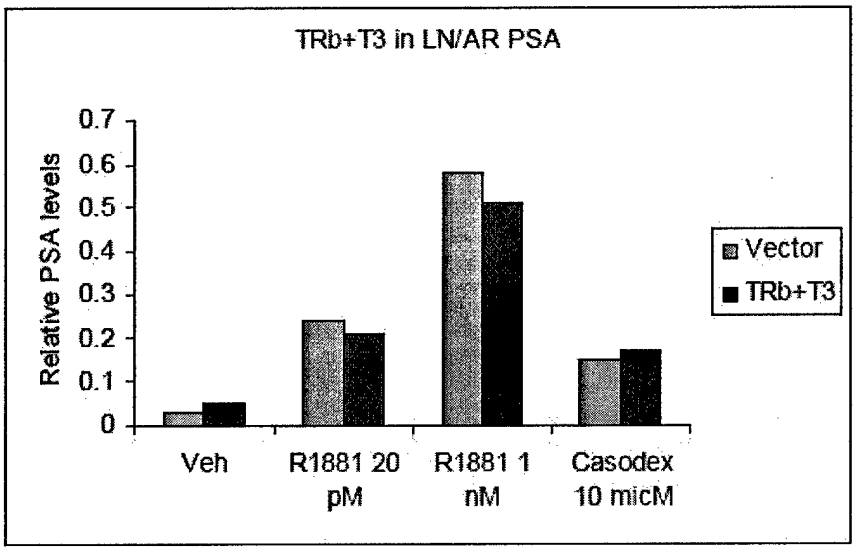

Figure 6. Unliganded or liganded TRβ effect on AR activity on an endogenous gene. A, LNCaP cells expressing control or TRβ were treated with different concentration of R1881 or 10 μM and secreted PSA was measured by ELISA. B, LN/AR cells expressing control or TRβ were treated with different concentration of R1881 or 10 μM of Casodex in the presence of T3, and secreted PSA was measured by ELISA.

We also hypothesized that liganded TRβ will titrated away coactivators and inhibit AR activity if the same coactivators for promoting AR activity are limited in HR prostate cancer. To test this hypothesis, we treated AR-overexpressed LNCaP cells (LN/AR) with different doses of R1881 or with Casodex, and measured secreted PSA expression by

ELISA. Addition of a TRβ ligand T3 to TRβ over-expressed LN/AR did not significantly inhibit PSA expression in both low and high concentrations of R1881, or in the presence of Casodex (Figure 6b). These results suggest that coactivators are not limited, or liganded TRβ and AR do not share the same coactivators in the progression of prostate cancer.

To determine if exogenous TRβ is functional, we measured the luciferase activity controlled by four tandem repeat of AR responsive elements (4ARE) in the presence or absence of T3. TRβ enhanced the AR activity in this assay and the enhancement was diminished in the presence of T3 (Figure 7), consistent with reported data. These data suggest that TRβ and AR share the same coactivators and corepressors in an episomal assay system.

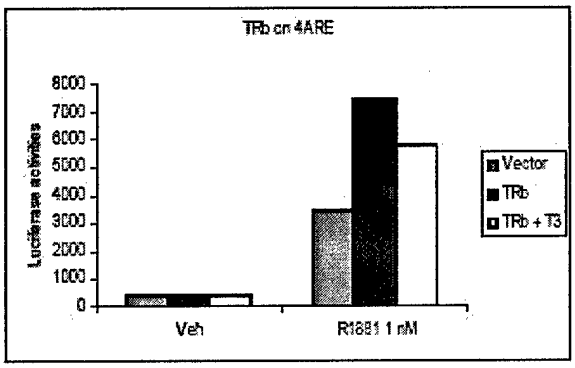

Figure 7. Unliganded or liganded TRβ affected AR transcriptional activity on an AR-driven exogenous luciferase reporter.

Taken together, we concluded that AR-regulated genes with native chromatin structures have different regulation than episomal constructs. They employ a different set of nuclear receptor coregulators.

Task 2. To examine cofactor complex changes when AR is overexpressed using chromatin immunoprecipitation (ChIP) assay to identify which cofactors are critical (8-16 months)

We proposed to use ChIP to identify cofactors critical in the progression of prostate cancer. In the first year, we had optimized sonication and PCR condition for ChIP assay to detect AR or Pol II associated with the PSA promoter. In both control LNCaP or LN/AR cells, AR associates with the PSA promoter in the presence of agonists such as R1881 or DHT (Figure 8, lanes R and D), and also in the presence of antagonist Casodex (Figure 8, lanes B). In the control LNCaP cells, Pol II associates with the PSA promoter only in the presence of agonists, but not in the presence of antagonist Casodex. However, in AR overexpressed cells, Pol II associated with the promoter not only in the presence of agonists, but also in the presence of antagonist. These results suggest that AR overexpression converts an antagonist to an agonist to recruit Pol II to an AR-regulated promoter.

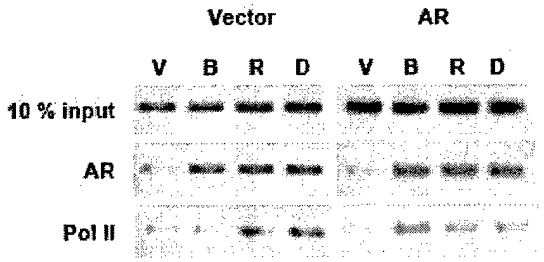

Figure 8. ChIP assay to detect AR and Pol II association with the PSA promoter in control (vector) or AR overexpressed LNCaP (AR) cells in the presence of vehicle (V), 10 μM of Casodex (B), 0.1 nM of R1881 (R), or 1 nM of DHT (D).

KEY RESEARCH ACCOMPLISHMENT

1. Demonstrated that blocking deacetylase activities by TSA or sodium butyrate inhibits AR transcriptional activity, measured by endogenous PSA expression.
2. Demonstrated that TRβ had no squelching effect on AR transcriptional activity, measured by endogenous PSA expression.
3. Developed ChIP assay to detect AR and Pol II associated with the PSA promoter.

REPORTABLE OUTCOMES

None

CONCLUSION

In the first year of study, we proposed to determine if chromatin remodeling or other functions of nuclear receptor cofactors are involved in the HR progression and if coactivators and corepressors are critical in this process. We unexpectedly discovered that blocking deacetylase activities inhibited AR activity, measured by PSA expression. This is in contrast to published data that deacetylase activities are transcriptionally inhibitory in exogenous reporter systems. We also demonstrated that TRβ had no squelching effect on AR transcriptional activity, measured by endogenous PSA expression. Finally, we had developed ChIP assay to detect AR and Pol II associated with the PSA promoter.